EFFECTS OF CRUDE OIL TREATED SEAWATER ON THE

METABOLISM OF PHYTOPLANKTON AND SEAWEEDS

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A

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By

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ABSTRACT

Prudhoe Bay crude oil was applied to natural populations of phytoplankton from Port Valdez, Alaska to examine its effect on photosynthesis. Studies were made at various times between July 1971 and June 1972, at oil concentrations ranging from about 0.001 - 10 ppm, and under various conditions of temperature, light and exposure time. Varying degrees of photosynthetic inhibition and stimulation were observed, apparently resulting from a complex interaction of physical, chemical and biological factors and the amount of oil present.

Inhibition of photosynthesis occurred at oil concentrations of 0.06 ppm or greater during June, with stimulation observed at about 0.003 ppm. Light levels above 0.2 ly/min, 25% of full natural light intensity in April, caused a reduction in photosynthesis at about 0.03 ppm. Variations in temperature affected the degree of oil toxicity to phytoplankton. At 1 - 2 ppm photosynthesis decreased with increasing temperature (5 - 20 C) in March while in April photosynthesis was optimum between 10 - 15 C. The species composition was significantly altered by a 48-hour exposure to oil, showing an increase in *Phaeocystis pouchetii* and a decrease in *Thalassiosira nordenskiöldii*.

The photosynthetic response of 8 species of seaweeds were tested at different oil concentrations. Both inhibition and stimulation of photosynthesis were observed. The green algae *Cladophora stimpsonii*, *Enteromorpha intestinalis* and *Ulva fenestrata* appeared to be most adversely affected by oil.

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1. INTRODUCTION

The problem of oil pollution of the sea has become increasingly more serious in recent years as greater amounts of crude oil and other petroleum products have been shipped over marine transportation routes to meet the energy demands of an ever-growing world population. The yearly oil influx into the sea, from all sources, is estimated to be between 3 - 10 million metric tons, 0.003% - 0.01% of the world's annual oil production (Ketchum 1972), which occurs primarily in the coastal regions, the most productive area of the ocean. In spite of an almost universal awareness of petroleum waste accumulations in the sea and the possible consequences of such accumulations to marine life, only recently has much been done on an international scale to mitigate these problems through combined scientific research or through governmental and industrial regulations on shipping and related activities of commerce. Future oil industry operations - drilling, transportation, refining - will see an increasing stringency in regulations concerning environmental pollution and the establishment of liabilities that will act to strongly discourage oil pollution at sea.

The emphasis in marine pollution research has often been placed upon the commercially important and aesthetically valuable species with little regard for the lower plants and animals comprising the plankton, upon which the higher forms either directly or indirectly depend. For example, studies have been made on oil toxicity to coho and pink salmon (J. E. Morrow, in press; E. Wolf, personal communication) without concurrent or supporting detailed studies on the food organisms required

by the fish. Finding that pink salmon fry suffer no adverse effects from crude oil pollution at concentrations as high as 10 ppm, for example, is of little consequence if the zooplankton upon which they feed show total mortalities at levels of 0.1 - 1.0 ppm.

Any human event which creates an imbalance in the community structure within an ecosystem, particularly if it occurs from damage to lower food chain organisms, risks unwanted and possibly permanent changes in that system. The proposed activities of the petroleum industry in Port Valdez, Alaska, the terminis of the proposed trans-Alaska oil pipeline from Prudhoe Bay on the Arctic Ocean, offer a potential threat to the stability and vitality of its ecosystem, and it is obvious that questions concerning the effect of petroleum hydrocarbons in that environment need answering before accurate assessments can be made and realistic management practices instituted. The oil companies have proposed a super-tanker facility at Jackson Point in Port Valdez which will not only be equipped to dock tankers for loading of crude oil to be shipped south, but will also have the capability of treating tanker ballast water to reduce the amount of hydrocarbon entering the Port waters.

Port Valdez is a relatively deep, narrow, glacial fjord located in the northeastern part of Prince William Sound at about 61°N latitude; it tends east-west approximately 12 miles in length and 3 miles in width, and is well protected and ice free the year around. The Institute of Marine Science at the University of Alaska has recently completed a one-year marine environmental baseline study of the Port. The results of this interdisciplinary study will soon be

published (Hood, Shiels and Kelley, in press) and will provide a base for future work. Concurrent with the physical, chemical and biological studies of other investigators, toxicity studies were undertaken to determine the effect of crude oil on the marine primary producers, phytoplankton and seaweeds.

The prime objective of the toxicity studies was to determine the effects of varying concentrations of crude oil on algal metabolism (i.e., photosynthesis and respiration) in the range expected to occur in the treated tanker ballast water (0.001 - 10 ppm).

A second objective was to study whether temperatures between 0 - 20 C would have different effects on phytoplankton metabolism at low crude oil concentrations. The treated ballast water is expected to vary as much ås 20 C from ambient seawater temperatures.

A third objective was to examine the effects of varying light intensities on algal metabolism at ambient temperature levels. Since the depth of the euphotic zone varies seasonally, as well as daily, and the depth of the ballast water outfall pipe remains fixed, it is possible that the ballast water could be injected into the euphotic zone.

A fourth objective was to test changes in phytoplankton metabolism over prolonged exposure periods to oil. The incubation periods routinely used in primary productivity experiments (2 - 6 hours) may not be long enough to allow the full damage of oil to occur. Also, oil-induced changes in the species composition of a phytoplankton community may occur given sufficient exposure time.

And finally, the fifth objective was to measure the effects of oil on metabolism of some of the predominant intertidal and shallow subtidal seaweed species.

2. METHODS

Prudhoe Bay crude oil (AFS-SR 101A, B.P. Alaska, Inc.) was used in toxicity experiments conducted on natural phytoplankton populations and on some major species of seaweeds during five cruises of the *R/V Acona* in Port Valdez, Alaska, and two subsequent field trips to the area. Certain modifications were made to methods and techniques as the study developed.

2.1 Preparation of Oil-treated Seawater

Crude oil was applied to marine algae in three ways: emulsion, volume additions and oil slick. In all cases the oil was at room temperature (~22 C) before it was added to seawater. When not in use it was stored frozen.

2.1.1 Oil-seawater emulsion

Preparation of 100% oil-saturated seawater involved 1) filtering raw seawater through 0.45 µm Millipore^{R} or glass fiber filters, 2) pasteurizing it at 60 C for 2 or more hours, 3) adding an excess of oil (i.e., at least 50 ml) to 2 liters of the seawater, 4) shaking (wrist-action shaker) for at least 24 hours in a tightly stoppered glass bottle, and 5) draining the water from the bottom of the bottle through a spout after the oil-seawater mixture had stood for 12 or more hours. This oil-treated seawater is referred to as oil-saturated seawater, and in the toxicity experiments was used full strength or serially diluted.

2.1.2 Volume additions of oil to seawater

Volume to volume additions of oil to seawater were prepared by 1) filtering and pasteurizing raw seawater, 2) adding a known volume of oil to a measured volume of seawater, 3) shaking the mixture for 24 hours and then using it in toxicity experiments immediately. This stock solution was diluted to half with raw seawater in some experiments, or was first serially diluted with filtered seawater and then diluted to half strength with raw seawater. The concentrations reported in the various experiments represent the amount of oil added *to* the seawater, and *not* necessarily dissolved *in* it.

2.1.3 Oil slick on seawater

Oil was added to the surface of seawater in incubation vessels in two types of experiments. In one, referred to as the "Dickman experiments" (Dickman 1971), 5 ml of oil was added to 125 ml of raw seawater. In the other, the species succession experiment, small volumes of oil (0.01 ml and 1.0 ml) were added to 1-liter samples of raw seawater.

2.2 Hydrocarbon Analysis

Quantitative analysis of oil-treated seawater used in phytotoxicity experiments was conducted independently by the Institute of Marine Science (IMS) and the Valdez facility of Battelle Northwest Laboratories.

2.2.1 Dissolved organic carbon method

Analysis of crude-oil samples at IMS employed the method of Menzel and Vaccaro (1964) which entails the oxidation of dissolved organic carbon (DOC) to CO_2 and its measurement by infrared spectrophotometric analysis. Collection of samples for DOC analysis was made by adding 5 ml of seawater to a 10-ml ampoule containing $K_2S_2O_8$, H_3PO_4 (3%) and distilled water, flushing with oxygen for 5 min and sealing the ampoule which had previously been baked at 575 C four or more hours and stored top to top connected by a short piece of clean Tygon^R. The organic carbon content in seawater was measured by autoclaving the sealed ampoule to oxidize the organic carbon, and then measuring the resultant CO_2 by passing it through a Beckman Model IR215 CO_2 Infrared Analyzer. Concentrations of carbon were converted to crude oil concentrations by assuming an average crude oil carbon content of 86% by weight (Hobson 1967).

2.2.2 Battelle method

Samples analyzed by Battelle were collected in pre-treated glass jars and extracted with carbon tetrachloride (CCl_4) within 1 hour after collection. Three extractions were made on each seawater sample, an aliquot of the combined CCl_4 fraction was placed in a KBr volatile liquid cell, and then read on a Beckman Model IR-20 Infrared Analyzer from 2400 to 4000 wave numbers. The intensity of the CH_2 bond stretching was recorded and compared to crude oil standards. This method is reported to reliably measure hydrocarbon levels as low as 1 ppm in seawater (E. Wolf, personal communication).

2.3 Collection and Identification of Algae

Phytoplankton standing stock was determined by collecting seawater samples in a polyvinyl chloride (PVC) bottle, preserving the samples with 4% formalin buffered with sodium acetate, and later counting by the Utermöhl inverted microscope technique (Utermöhl 1931). Qualitative sampling consisted of taking vertical tows from the bottom of the euphotic zone to the surface with a 0.5-m, no. 25 mesh net towed at 13 m/min. Samples were preserved with buffered formalin until species identifications were made using a Zeiss phase-contrast microscope. A detailed account of methods and results of the Valdez phytoplankton study is given by Horner *et al.* (In press).

Seaweed samples were collected near Jackson Point by hand (intertidal) or by dredge (subtidal), placed in fresh seawater and kept in subdued light until used in toxicity experiments. Identification of seaweeds was made at the Invertebrate and Marine Collection Center, University of Alaska Museum. Accession numbers are given in Table 1 for voucher specimens of all the seaweeds used in the oil toxicity experiments except *Enteromorpha intestinalis*.

2.4 Preparation of Plant Material

Plant material used in oil toxicity experiments was tested as soon after collection as possible; usually the time was less than an hour for phytoplankton and not more than four hours for the seaweed species.

Table 1: Species of seaweeds with accession numbers studied in crude oil toxicity experiments in Port Valdez, Alaska from July 1971 to May 1972.

Taxa	Accession No.
Chlorophyta	
Cladophora stimpsonii Harvey, 1859	50584
Enteromorpha intestinalis (L.) Link, 1820	-----
Ulva fenestrata Postels and Ruprecht, 1840	50583
Phaeophyta	
Alaria tenuifolia Setchell, in Collins, Holden and Setchell, 1901	50582
Costaria costata (Turner) Saunders, 1895	50580
Fucus distichus Linnaeus, 1753	50579
Laminaria saccharina (L.) Lamouroux, 1813	50581
Rhodophyta	
Rhodymenia palmata (L.) Greville, 1830	50586
Halosaccion glandiforme (Gmelin) Ruprecht, 1851	50585

2.4.1 Phytoplankton

Phytoplankton were usually collected in a 30-liter PVC, non-toxic sampling bottle (General Oceanics Inc., Model 1010). On a few occasions samples were obtained from the ship's PVC non-toxic seawater system which has an intake 2.5 m below the surface. In both instances the water sample was used as soon after collection as possible, taking precautions not to allow any changes in temperature or to expose it to intense light. During the summer and fall cruises toxicity experiments were carried out with net plankton. Plankton was collected with horizontal tows of a no. 25 mesh (0.048-mm) net at selected depths and the contents strained through a no. 0 mesh (0.571-mm) net to remove the larger zooplankton; the material which

passed through was suspended in fresh seawater and designated phytoplankton "soup".

2.4.2 Seaweeds

Marine macrophyte algae, or seaweeds, were collected from the intertidal and subtidal zones near Jackson Point, Port Valdez, with the exception of *Alaria tenuifolia*, which was collected in the Valdez small boat harbor and may not be indigenous to Port Valdez. This species was collected from the hull of the *R/V Ursa Minor*, which had been home-ported in Juneau for several years before being brought to Valdez in October of 1971 to serve as a floating laboratory. This species was selected because it was in an early growth stage. The fronds were from 6 - 9 cm in length, compared to a mature length of approximately 50 cm, and this allowed the whole frond to be used in toxicity experiments. Small pieces were cut from the fronds of *Costaria costata*, *Fucus distichus*, *Laminaria saccharina* and *Ulva fenestrata* because of their large sizes. *Cladophora stimpsonii*, *Enteromorpha intestinalis*, *Halosaccion glandiforme* and *Rhodymenia palmata* were used either as whole plants or single fronds, depending on the plant size. The plants were kept in a fresh supply of seawater in low light prior to use in experiments.

2.5 Measurements of Metabolism

Two methods were used to measure the effects of crude oil on photosynthesis and respiration by marine plants. The ^{14}C method was used in both seaweed and phytoplankton studies, employing Geiger-Mueller

(G-M) counting (Strickland and Parsons 1968) or the liquid scintillation counting (LSC) technique (Schindler 1966; Wolfe and Schelske 1967); and, in some seaweed experiments photosynthesis was measured by the light and dark bottle method (Strickland 1960) employing a modification of the Winkler oxygen titration method (Wallen and Hood 1968).

The techniques used to evaluate oil toxicity in these studies were taken in part from the work done by Hood, Duke and Stevenson (1960) and Strand *et al.* (1971).

2.5.1 Dissolved oxygen method

When using this method plant material was added to glass incubation bottles containing filtered seawater (control samples) or filtered seawater with crude oil (test samples). Bottles with no plant material were incubated along with bottles containing seaweed to correct for non-plant oxygen changes. After 4-hour incubation the plant material in each bottle was removed and dried at 105 C for 12 hours prior to being weighed, and the oxygen content of the seawater determined. Photosynthetic rates were computed from O_2 differences between light and dark bottles (Strickland 1960).

2.5.2 Carbon-14 method

Studies of oil toxicity using the ^{14}C method involved placement of raw seawater (containing phytoplankton) into either 125 ml clear glass or totally darkened reagent bottles, application of crude oil as specified by the particular experiment and the addition of 5 µc of $^{14}C\text{-}HCO_3^-$. The bottles were then stoppered and placed in a seawater-cooled incubator under natural light, or under artificial light at

ambient seawater temperatures. Incubation time ranged from 2.5 - 9 hours. After incubation the contents of each bottle were filtered through 0.45-μm Millipore^{R} cellulose-acetate filters and rinsed with 5 ml of 0.005 N HCl made with filtered seawater. The filters were then placed in ventilated plastic petri dishes, dried, and later mounted on aluminum planchets and counted on a Picker low-background β-counter.

The LSC technique was used in experiments conducted on *Acona* cruises 128 and 131, and on two subsequent field trips to Valdez. Instead of desiccating the filters, they were placed directly into scintillation vials containing 10 ml of Aquasol^{R} (NEF-934, New England Nuclear, Boston, Mass.), a scintillation cocktail mixture. After 12-24 hours the filters became nearly transparent and if the contents were shaken several times within 1 - 2 days of filter addition, the filter remnants were negligible. This was advantageous because any suspended matter interferes with accurate ^{14}C counting. After addition of 2.3 ml distilled water to form the gel, the samples were ready for counting.

Seaweed photosynthesis was also tested with the ^{14}C method. The experimental procedure was similar to that used for phytoplankton, except only the LSC technique of counting was used. In seaweed studies, filtered, pasteurized seawater (oil-treated and untreated) was added to 125-ml reagent bottles, one light and one dark bottle for each oil concentration plus a light and a dark bottle for the controls, followed by the addition of healthy seaweeds (either a portion of, or the entire plant). Two ml of seawater were removed and 5 μc ^{14}C-HCO_3^- added.

Incubation ranged from 2 - 4 hours in a seawater-cooled incubator under natural light. The bottles were removed and shaken at half-hour intervals. Following incubation the plant material was removed, rinsed first in filtered seawater and then in dilute acid (0.005 N HCl), blotted dry, and placed in glassine envelopes. In the laboratory, the dried seaweed samples were ground using a mortar and pestle and weighed, or were weighed and then digested in a mixture of Protosol^{R} (New England Nuclear, Boston, Mass.) and NCS^{TM} (Amersham/Searle Corp., Arlington Heights, Ill.) before being treated with Aquasol^{R}. The contents in each scintillation vial were mixed by shaking and then allowed to stand for at least 48 hours before addition of distilled water.

Scintillation vials containing the phytoplankton or seaweed material were wiped clean and counted in a Nuclear Chicago Model 6848 Liquid Scintillation System. The external standard mode of operation, using Barium-133 as the gamma source, was used for quench measurement as opposed to the channels ratio mode. Quenched standards were made up in Aquasol^{R} using CCl_4 as the quenching agent. The external standard ratio (ESR) and corresponding counting efficiency (EFF) value for each quenched standard was plotted and a standard curve drawn.

After plant samples were counted, ESRs were calculated and the corresponding EFFs taken from the standard curve. Since the filters often contained relatively large quantities of oil, color quenching became significant. Chlorophyll *a* and other plant pigments also

produced color quenching when experiments were conducted with high phytoplankton standing stocks or seaweeds. This reduced the counting efficiency. With a reliable quenched standard curve, however, an accurate EFF value may be obtained for each sample; and, if EFF plus background-corrected counts per minute (CPM) are known for the sample, sample activity in disintegrations per minute (DPM) may be determined. Such a quenched standard curve was used to obtain reliable ^{14}C activity measurements.

2.6 Statistical Analysis

The statistical tests applied to the data from various oil toxicity experiments followed those described by Snedecor (1962).

3. RESULTS AND DISCUSSION

Prudhoe Bay crude oil was used in toxicity experiments on five cruises of the *R/V Acona* in Port Valdez (*Acona* cruises 117, 122, 125, 128 and 131) and during two field trips in spring and summer to determine the concentrations of oil in seawater that caused a depressed photosynthetic rate (i.e., were toxic) in natural marine phytoplankton populations under differing temperature, light intensities and exposure times. The toxicity of crude oil at several concentrations to 8 species of predominant intertidal and subtidal seaweeds was also tested.

Analytical determinations of oil content in seawater were made in most experiments. However, where direct measurements were not made, the concentrations were estimated based on known oil content of similarily prepared oil-treated seawater.

The results of the experiments are presented as differences in carbon assimilated, that is, CPM or DPM per volume of seawater or g dry wt, or oxygen liberated (ml $O_2 \cdot$g dry wt^{-1}). Pertinent data from toxicity experiments in which the ^{14}C method was used are tabulated in Appendix D.

Crude oil toxicity experiments in this study can be generally described as follows. Low concentrations of fresh (unweathered) crude oil were applied to natural populations of marine phytoplankton or seaweeds for relatively short incubation or exposure times, under simulated natural light and temperature conditions. Photosynthesis was usually estimated by the ^{14}C method in experiments conducted in the field.

Previous studies on the effects of oil pollution on marine algae are difficult to compare to this study. Some of the difficulties result from 1) treatment of the algae with substances other than crude oil (bunker oil, diesel oil, and other refined petroleum products), 2) treatment of the algae with crude oil plus a dispersant chemical, 3) differences in method of applying oil (emulsion, surface film, aqueous phase of oil-seawater mixture), 4) the amount of oil used, 5) how algal metabolism was reported (primary productivity, cell counts, dehydrogenase activity), 6) test organism(s) used (unialgal, axenic cultures, mixed populations) and, 7) the location and nature of testing (laboratory, field, *in situ* or chemostatic).

3.1 Effects of Oil on Phytoplankton

Phytoplankton growth response to crude oil contamination is difficult to accurately assess in ecological studies where attempts are made to simulate the natural environment. Even in controlled laboratory experiments it is difficult to determine if an observed change in phytoplankton metabolism is due directly to oil contamination or results from a combination of other factors such as light, temperature, salinity, and dissolved nutrients superimposed upon the effects of crude oil.

The main difficulty in an ecological study using natural populations of phytoplankton is that species abundances differ with the time of year. A study of temporal changes in phytoplankton toxicity levels, therefore, might reflect only the changes in species composition in a population and their responses to crude oil rather than changes due to seasonal

variations of natural physico-chemical events. Each experiment conducted in this study, therefore, should be evaluated on its own, considering all environmental parameters. Toxicity tests conducted with indigenous phytoplankton during the spring bloom, for example, under natural conditions of light, temperature, and water chemistry would perhaps be of greater value in predicting consequences of crude oil pollution in the marine ecosystem than studies with monocultures of plankton algae in laboratory chemostats. The value of the laboratory studies is in its ability to provide information emphasizing the mechanisms rather than the immediate consequences of oil toxicity.

3.1.1 Oil concentration and toxicity

Toxicity experiments were carried out to determine the effects of varying concentrations of crude oil on phytoplankton photosynthesis in the range expected to occur in treated tanker ballast water (0.001 - 10 ppm).

December experiment: During December (*Acona* cruise 125) nutrient concentrations were relatively high (e.g., NO_3^--N = 6 µg-at·liter^{-1}), phytoplankton primary production (0.2 mg C·m^{-3}·hr^{-1}) and standing stocks (0.2 mg Chl *a*·m^{-3}) were low. The most common species of phytoplankton in the water were small unidentified flagellates and the diatoms *Nitzschia closterium*, *Rhizosolenia stolterfothii* and *Skeletonema costatum*.

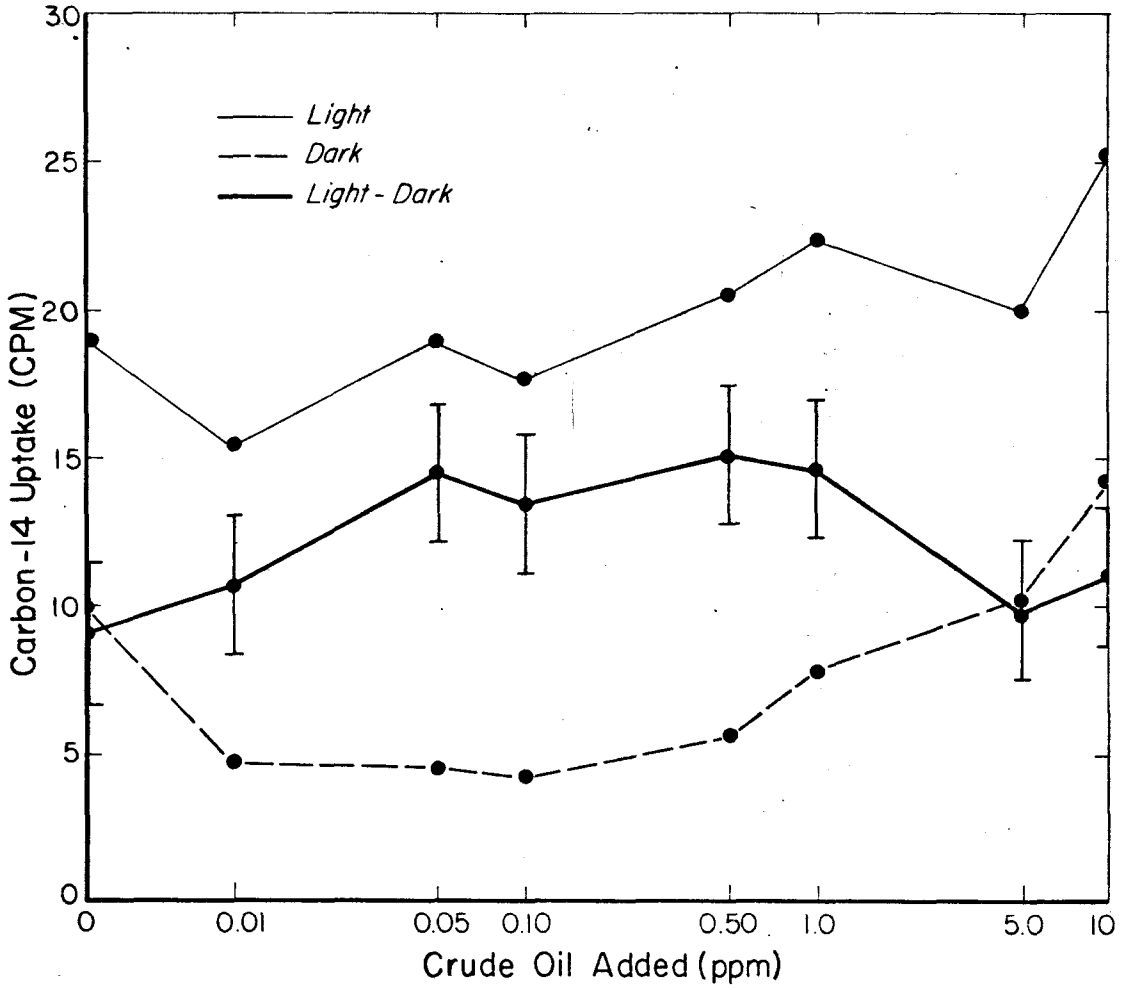
Seawater was collected from the *Acona's* non-toxic seawater system and incubated for 6 hours at sea-surface temperatures under artificial light. Light intensity approximated the average maximum natural level,

0.10 ly/min. The estimated concentration of the 10 ppm (v/v) oil-seawater mixture was between 0.8 - 2.0 ppm; a mean of 1.4 ppm was assumed.

A one-way analysis of variance was performed on the December experiment data (Fig. 1), and demonstrated a significant oil concentration effect with respect to phytoplankton photosynthesis ($P = 0.05$). Dark values were assumed to be representative and were subtracted from each of the three corresponding light values for each oil concentration. The 95% confidence limits were than calculated for points on the light-minus-dark curve. Phytoplankton photosynthesis was found not to differ significantly between the control and 1.4 ppm sample (Fig. 1). However, at concentrations between 0.007-0.14 ppm it was significantly different from the control and 1.4 ppm samples, showing a 50% stimulation in photosynthetic rate at about 0.05 ppm of oil.

March and April Dickman experiments: Four experiments were conducted during March (*Acona* cruise 128) and one during April (*Acona* cruise 131) following the procedure outlined by Dickman (1971) for testing the effects of crude oil on phytoplankton photosynthesis. During March nutrients were high (e.g., NO_3^--N = 20 µg-at·liter^{-1}) and standing stock was low (0.1 mg Chl a·m^{-3}). In contrast, nutrient levels during April were low (NO_3^--N <1 µg-at·liter^{-1}), but standing stocks were very high (∿9 mg Chl a·m^{-3}). The species composition also differed markedly between the two periods. In March small flagellates were abundant with some diatoms present, predominantly *Nitzschia closterium*, and in April the chrysophyte *Phaeocystis pouchetii*,

Figure 1: Photosynthesis by phytoplankton incubated for 6 hours under artificial light and natural seawater temperature in relation to additions of crude oil during December 1971 in Port Valdez, Alaska. (For actual crude oil concentrations see text.) Means of three replicate determinations and their 95% confidence limits are presented for the light-minus-dark values.


Light
Dark
Light - Dark
Carbon-14 Uptake (CPM)
30
25
20
15
10
5
0
0
0.01
0.05
0.10
0.50
1.0
5.0
10
Crude Oil Added (ppm)

the choanoflagellate *Monosiga marina* and the diatoms *Thalassiosira nordenskiöldii*, *Fragilariopsis* sp. and *Chaetoceros debilis* were the typical forms.

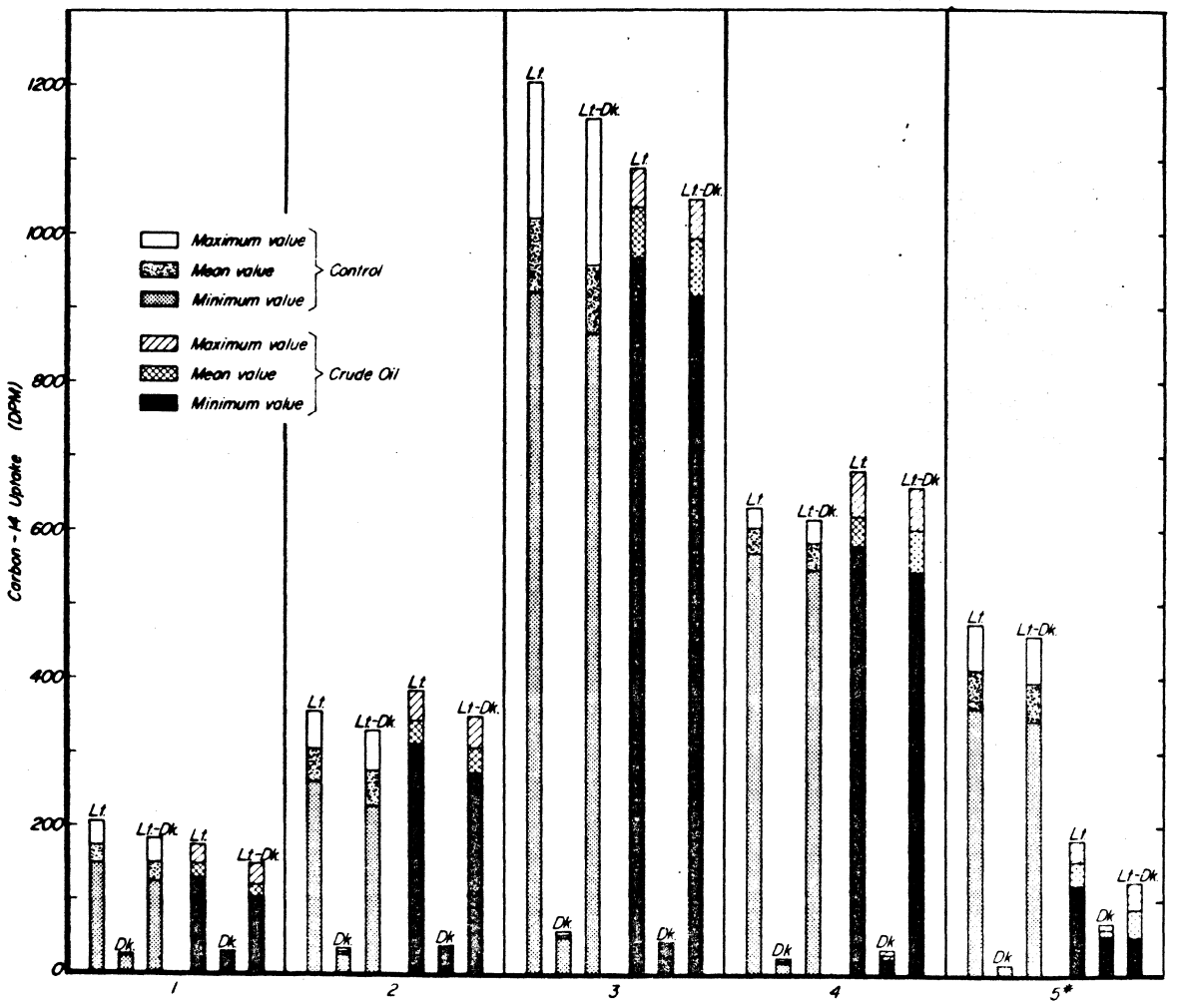
In each experiment 5 light and 2 dark bottles were used for both the control and oil-treated sets. Bottles were filled with raw seawater collected from about 2.5 m near Jackson Point, except in the third March experiment when seawater was collected in the Valdez small boat harbor. After inoculation with ^{14}C (both sets) and oil (oil-treated set) the bottles were incubated 4 hours under natural light and sea-surface temperature in a seawater-cooled incubator.

Samples collected in the Valdez boat harbor indicated a higher photosynthetic rate than the three samples from Jackson Point (Fig. 2). All four March experiments, however, indicated no statistical oil inhibition of photosynthesis. During April, photosynthesis was greatly decreased (about 80%) in oil-treated samples.

April experiment: During the spring phytoplankton bloom (*Acona* cruise 131) the chrysophyte *Phaeocystis pouchetii*, the choanoflagellate *Monosiga marina* and the diatoms *Fragilariopsis* sp., *Thalassiosira nordenskiöldii* and *Chaetoceros debilis* were the predominant species in Port Valdez waters. Primary productivity and standing stock conditions are indicated above.

Seawater was collected from 5 m near Jackson Point and incubated 2.5 hours under natural light and sea-surface temperature in a seawater-cooled incubator. Oil concentration measured by the DOC method was 3.1 ppm in the 50% oil-seawater mixture. Stimulation was again

Figure 2: Photosynthesis by phytoplankton incubated for 4 hours under natural conditions of light and temperature in relation to crude oil added (5 ml/125 ml) in four Experiments conducted during March (1 through 4) and one in April (5) in Port Valdez, Alaska. DPM values for Experiment 5 are 10^{-1} of actual.


Carbon - 14 Uptake (DPM)
1200
1000
800
600
400
200
0
Maximum value
Mean value
Minimum value
Control
Maximum value
Mean value
Minimum value
Crude Oil
Lt
Lt-Dk
Dk
1
2
3
4
5*

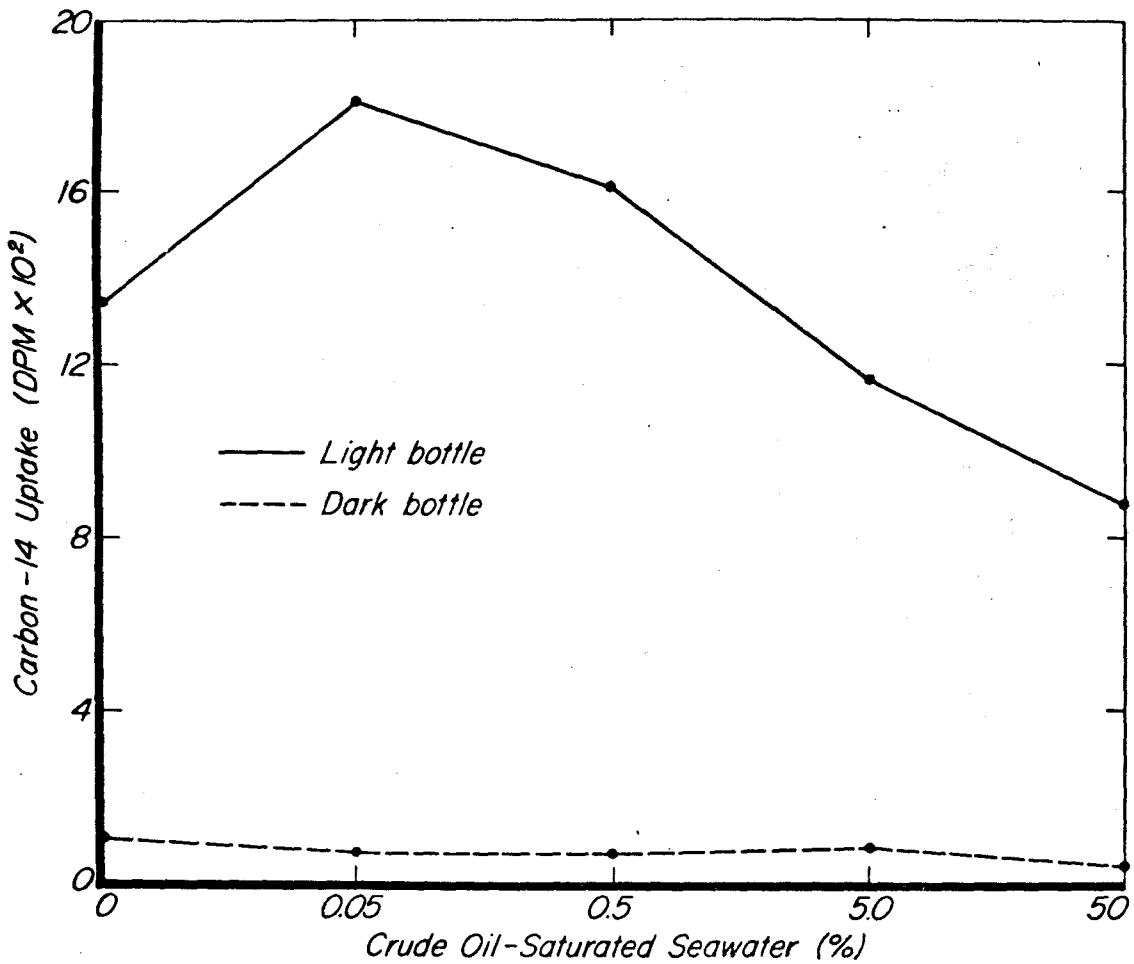
apparent at a concentration of about 0.003 ppm, representing about a 30% increase in photosynthesis (Fig. 3). Inhibition occurred at concentrations greater than about 0.2 ppm, and was about 35% at 3.1 ppm crude oil.

Carbon-14 uptake values for all oil-treated samples represent single determinations; control values are the means of duplicates. Assuming that similar errors are associated with oil-treated and control samples and that data points on figures 3 and 4 are representative for oil-treated samples, the error associated with each point was estimated. The coefficient of variation for oil-treated and control sets in the Dickman experiments (Fig. 2), where 5 replicates were tested for each set, did not differ significantly ($P = 0.05$). On this basis the 95% confidence limits for the April and June experiments (Figs. 3 and 4) are ±6% and ±19% respectively.

June experiment: During summer (mid-June) phytoplankton species composition and nutrient levels were different from those encountered in spring. Productivity was about 25% higher during June than in April. Nutrient concentrations were very low (e.g., nitrate was 0.7 $\mu g\text{-at} \cdot liter^{-1}$ as compared to 20 µg-at in March). The numerically dominant phytoplankter was the diatom *Thalassiosira decipiens* (90%), with the diatoms *Nitzschia* sp. and *Chaetoceros* sp. also present.

Seawater samples were collected from 2 m near Jackson Point, diluted to one half with filtered seawater (controls) or an oil-seawater mixture, and incubated 4 hours at sea-surface temperature (12 C) under natural light. The phytoplankton were subjected to a wide range of crude oil

Figure 3: Photosynthesis by phytoplankton incubated for 2.5 hours under natural conditions of light and temperature in relation to dilutions of seawater saturated with crude oil during April 1972 in Port Valdez, Alaska.

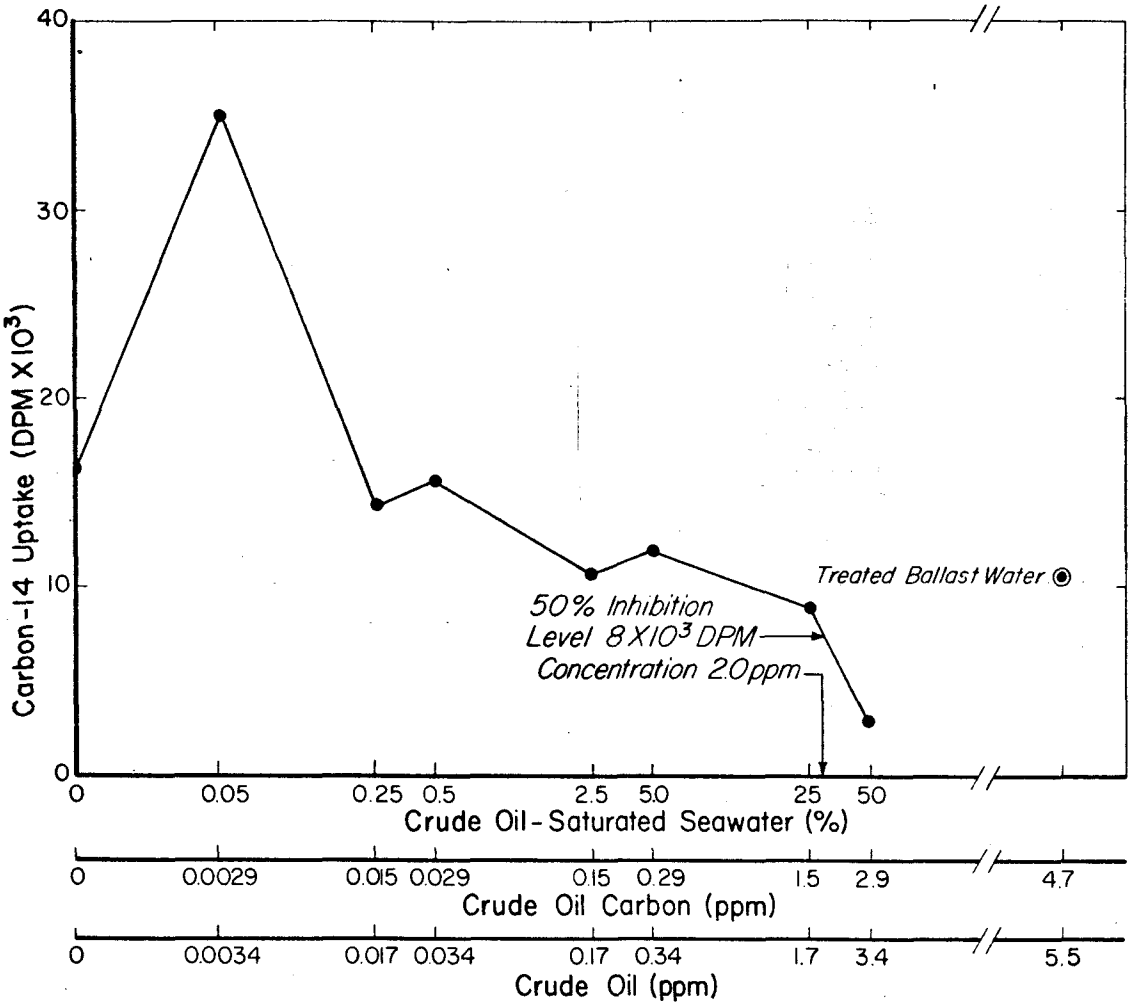

20
16
12
8
4
0
Carbon-14 Uptake (DPM x 10²)
Light bottle
Dark bottle
0
0.05
0.5
5.0
50
Crude Oil-Saturated Seawater (%)

concentrations and to simulated treated tanker ballast water furnished by the Battelle Northwest Laboratories field station at Valdez. The concentration of crude oil was 3.4 ppm in the 50% oil-seawater mixture and 5.5 ppm in the treated ballast water after 50% dilution with raw seawater (Fig. 4).

Stimulation of photosynthesis, about double the control, again occurred at a concentration of 0.003 ppm. Oil concentrations greater than about 0.06 ppm had an inhibitory effect on photosynthesis, and at 3.4 ppm inhibition was about 80%. The level of 50% inhibition (i.e., the oil concentration at which photosynthesis was reduced to one-half) was about 2 ppm. The toxicity of crude oil in treated ballast water was about one-tenth that of fresh crude oil. That is, about the same low photosynthetic rate occurred when ballast water crude oil was at 5.5 ppm and fresh crude oil at 0.67 ppm.

Analysis of crude oil by the DOC method gave concentrations of 1.2 - 1.6 ppm organic carbon in untreated seawater (i.e., ambient organic carbon in filtered seawater). These relatively high DOC values possibly resulted from increased phytoplankton activity during the spring phytoplankton bloom. The oil-saturated seawater mixture contained between 6.1 - 7.1 ppm *carbon*, or 4.5 - 5.9 ppm *crude-oil carbon*. Since crude oil is about 86% carbon by weight (Hobson 1967), the concentration was 5.2 - 6.9 ppm *crude oil*. Analysis of the same oil-saturated seawater by Battelle gave 7.5 ppm crude oil; the hydrocarbon content of the untreated seawater was negligible. The concentration of crude oil in

Figure 4: Photosynthesis by phytoplankton incubated for 4 hours under natural conditions of light and temperature in relation to dilutions of seawater saturated with crude oil during June 1972 in Port Valdez, Alaska.


40
30
20
10
0
Carbon-14 Uptake (DPM X 10³)
Treated Ballast Water
50% Inhibition
Level 8 X 10³ DPM
Concentration 20ppm
0
0.05
0.25
0.5
2.5
5.0
25
50
Crude Oil-Saturated Seawater (%)
0
0.0029
0.015
0.029
0.15
0.29
1.5
2.9
4.7
Crude Oil Carbon (ppm)
0
0.0034
0.017
0.034
0.17
0.34
1.7
3.4
5.5
Crude Oil (ppm)

the oil-saturated mixture was assumed to be 6.8 ppm (3.4 ppm for the 50% mixture), the mean of values obtained by the two methods. The treated ballast water was analyzed for crude oil content by Battelle and found to contain 10.9 ppm, or 5.5 ppm after 50% dilution with raw seawater.

Discussion: Crude oil was increasingly toxic to phytoplankton from December through June. The oceanographic conditions were quite different during each period of investigation: temperature, water chemistry, solar radiation, and species composition varied markedly (see Appendices A, B and C), and it is quite likely that some or all of these environmental factors account for the differences in magnitude of inhibition or stimulation.

Crafts and Reiber (1948) reported that peroxides and acids form in oils exposed to light and that both substances cause acute plant injury. The mean amounts of maximum daily light intensity measured for seven-day periods in Valdez were 0.10 ly/min for December, 0.53 ly/min for March, and 0.98 ly/min for April. If light intensity was a factor in toxicity, then April experiments should have shown greater toxicities than December and March experiments at equal oil concentrations, as suggested by the data. This does not preclude other factors such as temperature, nutrients, etc., from affecting toxicity levels.

Photosynthetic stimulation at low oil concentrations was observed in three experiments (Figs. 1, 3 and 4), and has also been observed by other investigators. Galtsoff *et al.* (1935) found that the diatom *Nitzschia closterium*, later reclassified as *Phaeodactylum tricornutum* (Lacaze 1969), showed growth stimulation (by cell counts) when treated

with the aqueous extract of a 12% mixture of crude oil and seawater. In studying the effects of crude-oil oil-dispersant mixtures on natural phytoplankton populations, Strand *et al.* (1971) found a significant increase in the photosynthetic rate at a 1 ppm oil concentration after an 8 hour incubation. The inconsistency between oil concentrations which resulted in stimulation (1 ppm vs. 0.003 ppm in this study) may be due to the absence of tests at concentrations lower than 1 ppm in Strand's work, the difference in incubation time (8 vs. 4 hours), and the method by which the crude oil concentrations were determined. The mechanism of stimulation of algal photosynthesis at low concentrations is unknown. It is perhaps due to transition metals in crude oil acting as micro-nutrients at very low concentrations (Hufford 1971).

Treated ballast water appeared to be less toxic than fresh crude oil. It is not known whether the treatment process "de-toxifies" the oil or if the process of simulating the ballast water (i.e., shaking and venting for several days) is the cause. Only treated ballast water was tested. Although there is no empirical evidence to explain the lower toxicity of ballast water, some suggestions are offered. The ballast treatment process removes about 75% of the paraffins and 55% of the aromatics (C_1 - C_{10} fraction) from simulated tanker ballast water without chemically changing or concentrating any of the hydrocarbon components (D. E. Brandon, personal communication; Table 2). Aromatics are generally considered the most phytotoxic components of crude oil, with the straight-chain paraffins least toxic (Currier and Peoples 1954).

The removal of a large percentage of aromatics as well as paraffins in the C_1 - C_{10} range would likely mitigate the toxic effects of crude oil. In addition, a significant loss of volatiles may occur from the oil in tanker ballast, provided the tanks are vented in transport. Smith (1968) reported that up to 25% by weight of crude oil spilled on the sea surface may evaporate within a few days after release. The loss of volatiles with concomitant auto- and bio-oxidation could significantly alter the composition of crude oil in tanker ballast water. The rate of change would depend upon temperature, available oxygen, degree of agitation, the physical state of the oil, and on the water chemistry (Pilpel 1968).

Table 2. Results of gas partitioning analyses of simulated Puget Sound ballast water containing Prudhoe Bay crude (C_1 - C_{10}). (Analyses performed by D. Johnson of Esso Production Research Company, Houston, Texas)

Hydrocarbon Names	Before Treatment (ppb)	After Treatment (ppb)
1. i-C_4	0.06	Trace
2. n-C_4	0.32	0.03
3. n-C_5	2.70	0.60
4. n-C_6	6.20	1.40
5. n-C_7	9.44	2.65
6. n-C_8	0.05	0.05
7. n-C_9	0.42	0.14
8. Benzene	4,069.00	1,729.00
9. Toluene	4,069.00	2,034.00
10. meta and para-Xylene	1,627.00	498.00
11. ortho-Xylene	559.00	290.00
12. C_9 Aromatics	765.00	285.00
13. C_{10} Aromatics	173.00	204.00
Total	11,281.19	5,044.87

3.1.2 Temperature and oil toxicity

Phytotoxicity experiments were conducted to study whether tempera-

tures between 0 - 20 C affect phytoplankton metabolism at low crude oil concentrations. These studies were undertaken because treated ballast water at times is expected to vary 20 C from ambient Port Valdez seawater.

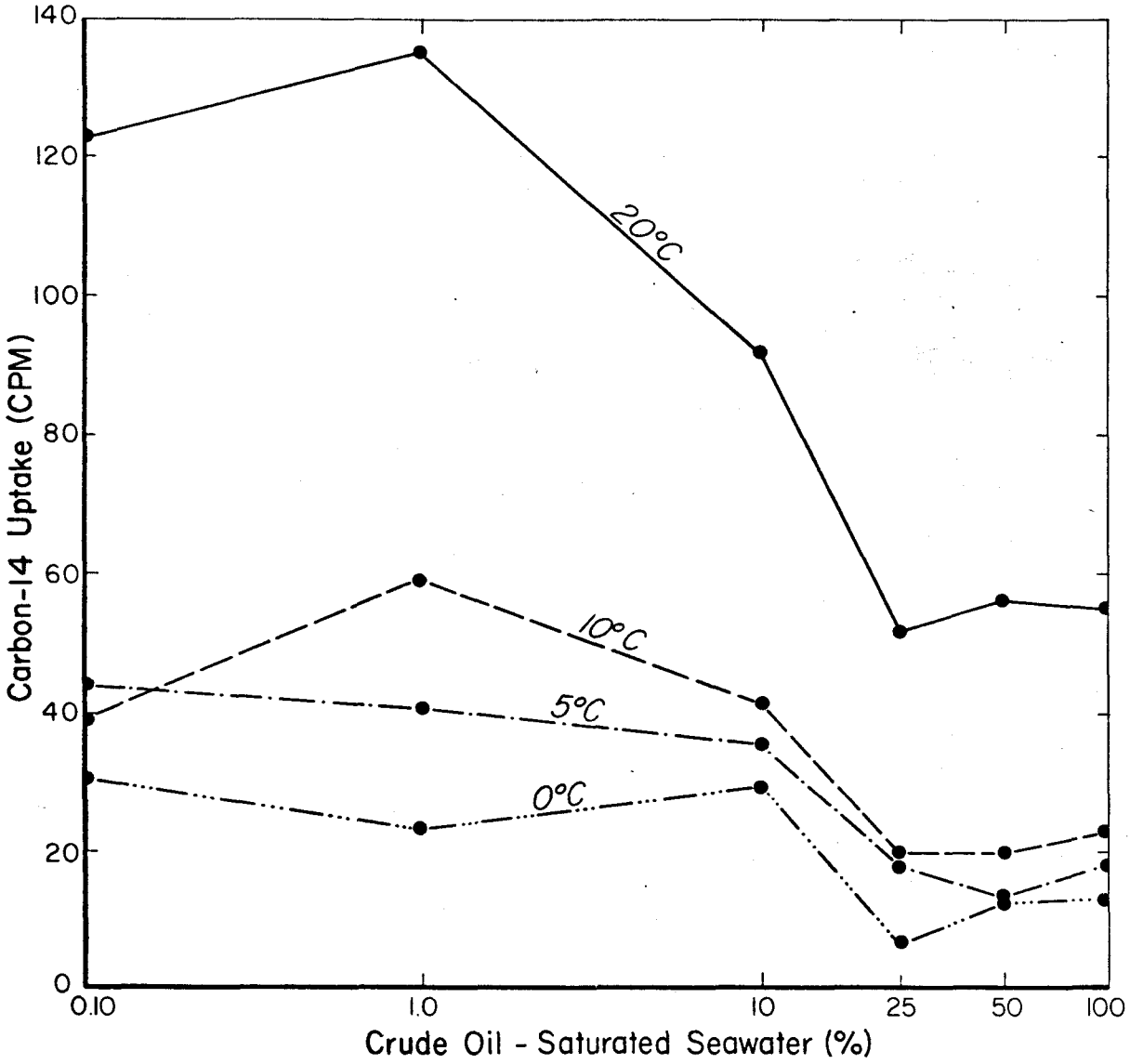
August experiment: During August (*Acona* cruise 117) nutrient levels, phytoplankton primary production and standing stock were moderately low (e.g., 2 µg-at NO_3^--N•liter^{-1}, 2 mg C•m^{-3}•hr^{-1} and 0.2 mg Chl *a*•m^{-3}). Dinoflagellates dominated the phytoplankton, with the genera *Ceratium* and *Peridinium* most predominant.

Reagent bottles were inoculated with phytoplankton "soup" (net plankton) and incubated for 6 hours under natural light at different temperatures and oil concentrations. The oil concentration of the 100% oil-seawater mixture was estimated to be 10 - 12 ppm (Fig. 5).

Precise temperature control was not maintained; however, an increase in photosynthesis with increased temperature was indicated at each oil concentration tested. Although it cannot be shown statistically, there is a suggestion that the greatest inhibition occurred not at the 100% but at the 25% concentration. This same trend was noted in an experiment with *Fucus distichus* (see Fig. 12).

October experiment: During October (*Acona* cruise 122) nutrient concentrations were low (e.g., 1 µg-at NO_3^--N•liter^{-1}), and primary production and standing stock were moderately low (2 mg C•m^{-3}•hr^{-1} and 0.5 mg Chl *a*•m^{-3}). Predominant phytoplankton species were *Nitzschia closterium, Skeletonema costatum, Leptocylindrus danicus* and *Thalassiosira gravida*.

Figure 5: Photosynthesis (light values only) by phytoplankton incubated for 6 hours under natural light conditions in relation to temperature and dilutions of seawater saturated with crude oil during August 1971 in Port Valdez, Alaska.


140
120
100
80
60
40
20
0
Carbon-14 Uptake (CPM)
20°C
10°C
5°C
0°C
0.10
1.0
10
25
50
100
Crude Oil - Saturated Seawater (%)

Reagent bottles containing an oil-seawater mixture were inoculated with phytoplankton "soup" and incubated for 9 hours under natural light at temperatures of 5, 6 and 10 C (Table 3). The oil concentration in the seawater was not measured, but the mixture was 10 ppm (v/v) or about 9 ppm (w/v) since Prudhoe Bay crude oil has a specific gravity of 0.893 (Thompson *et al.* 1971). The data suggest that an increase in temperature resulted in an increase in oil toxicity between 5 C and 10 C, with an 80% inhibition at 10 C - an average inhibition of 8.3% per degree C.

Table 3. Photosynthetic inhibition (light values only) for net plankton incubated under natural light conditions for 9 hours in relation to temperature at a crude-oil concentration of 9 ppm (oil added) during October 1971 in Port Valdez, Alaska.

Sample	Temperature (±1 C)	CPM	Crude Oil Inhibition %	Crude Oil Inhibition % $(°C)^{-1}$
Control	5	326	42	8.4
Oil	5	136		
Control	6	294	50	8.3
Oil	6	148		
Control	10	671	80	8.1
Oil	10	125		

March experiment: During March (*Acona* cruise 128) nutrient levels were very high (e.g., 20 µg-at NO_3^--N•liter^{-1}) and primary productivity and standing stock were 0.2 mg C•m^{-3}•hr^{-1} and 0.1 mg Chl *a*•m^{-3} respectively. Most of the phytoplankton cells were small flagellates; *Nitzschia closterium* was the predominant diatom.

Seawater samples were mixed half and half with either a 20 ppm oil-seawater mixture or filtered seawater, and incubated for 3 hours under artificial lights at different temperatures. The experimental oil concentration was 1.3 - 3.6 ppm.

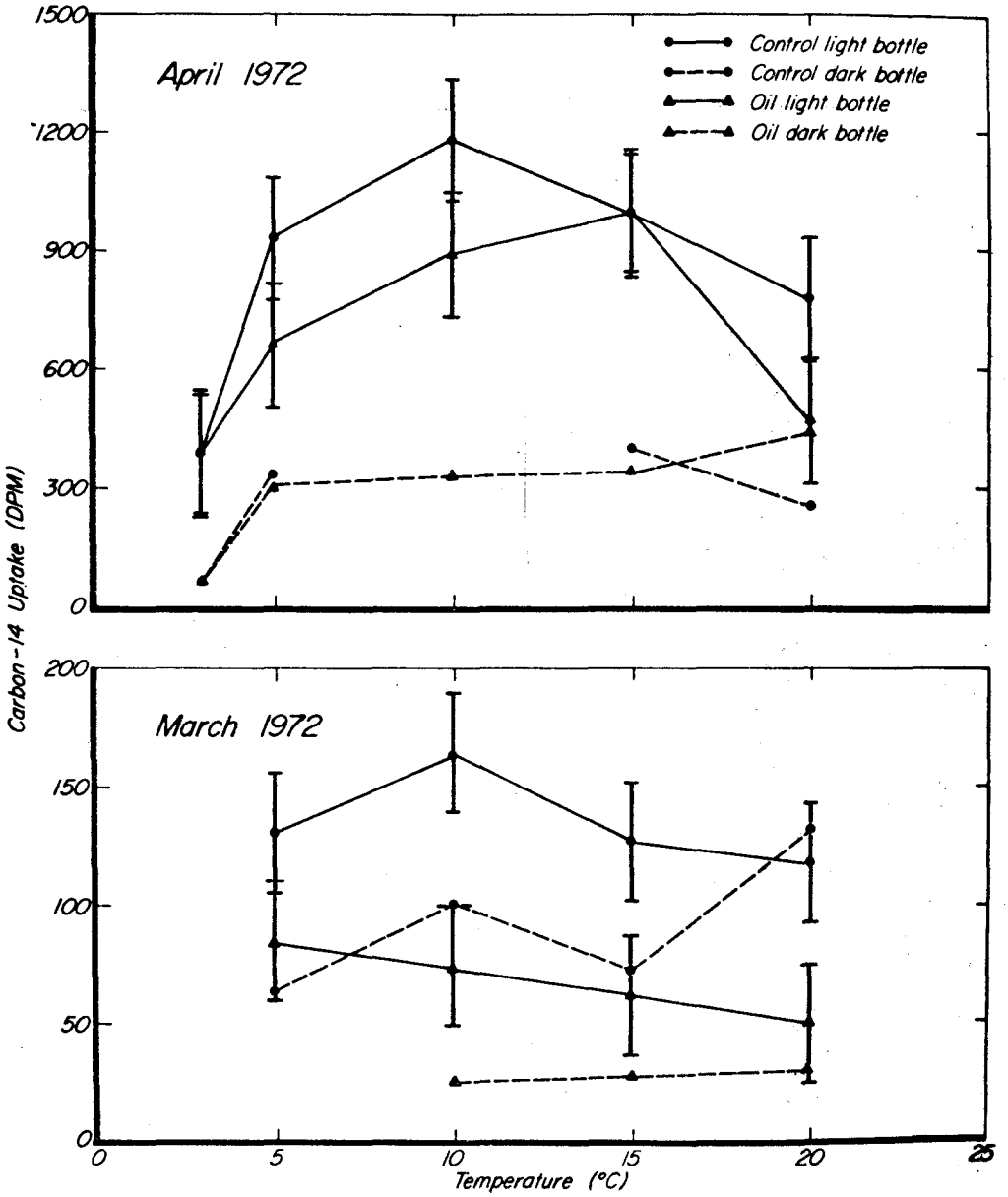
Controls indicated a 10 C photosynthetic optimum with zero net photosynthesis (light-minus-dark uptake) at 20 C (Fig. 6, bottom). Oil-treated samples suggested an inverse relationship between temperature and photosynthetic rate, that is, decreased photosynthesis with increased temperature.

April experiment: During April (*Acona* cruise 131) nutrient levels were quite low, with NO_3^--N occurring at <1 μg-at•liter^{-1}. Primary productivity and standing stock were 20 mg C•m^{-3}•hr^{-1} and ∿9 mg Chl *a*•m^{-3} respectively. Predominant species of phytoplankton were *Monosiga marina, Phaeocystis pouchetii, Chaetoceros debilis, Fragilariopsis* sp. and *Thalassiosira nordenskiöldii.*

Seawater samples were treated identical to the March experiment. The oil concentration was also the same (1.3 - 3.6 ppm). In this experiment (Fig. 6, top), the control samples again indicated a 10 C photosynthetic optimum. A 10 C or possibly higher optimum is also suggested for the oil-treated samples with net photosynthesis decreasing to near zero at 20 C. The rate of photosynthesis was similar for control and oil-treated samples at 3 C, the ambient seawater temperature.

Discussion: Phytoplankton treated with crude oil showed maximum carbon uptake (light values) at 5 C, 10 - 15 C and 20 C during March, April and August respectively, at oil concentrations between 1 - 4 ppm,

Figure 6: Photosynthesis by phytoplankton during March 1972 (bottom) and April 1972 (top) in relation to temperature and at a crude oil concentration of 1.3 - 3.6 ppm in Port Valdez, Alaska. Means of three replicate determinations and their 95% confidence limits are presented for light bottle values.


April 1972
March 1972
Control light bottle
Control dark bottle
Oil light bottle
Oil dark bottle
Carbon-14 Uptake (DPM)
Temperature (°C)
1500
1200
900
600
300
0
200
150
100
50
0
5
10
15
20
25

reflecting differences in phytoplankton species composition, as well as temperature, light, and possibly, nutrient levels.

The toxicity experiments in this study were all carried out in closed systems. This was particularly significant in the temperature experiments, where at higher temperatures more low-boiling petroleum fractions were retained than normally would have been in seawater (Pilpel 1968); these low-boiling fractions are known to cause plant injury when present in sufficient quantities (Currier and Peoples 1954). Because the loss of volatiles seems improbable in a closed system, serious errors may occur if efforts are made to relate these studies to the natural environment. In the case of treated tanker ballast water, however, the results from closed-system experiments may not differ considerably from natural systems, since the depth of treated ballast water entry into the Port is planned to be 15 m or greater (Hood *et al.*, in press).

No literature was found on temperature effects on oil toxicity to marine algae. However, since metabolic rate is proportional to temperature within the temperature tolerance range of a species, an increased rate of metabolism might lead to death more rapidly. Another possible explanation of oil damage to algae is the solubilization of the lipid portion of the plasma membrane, a process which is enhanced by increasing temperatures (Van Overbeek and Blondeau 1954). Also, the solubilities of the various components of crude oil in seawater are probably temperature dependent. And finally, the formation of toxic free radicals (e.g., -COOH, -OH, -CHO and -COR)

in crude oil is enhanced at higher temperatures by the process of auto-oxidation (Pilpel 1968).

3.1.3 Light intensity and oil toxicity

The depth of the euphotic zone is variable and in Port Valdez the factors most heavily affecting it are suspended sediment load and phytoplankton. Therefore, it is not only the seasonal changes in solar radiation which determine light intensity at a particular depth beneath the sea surface, but the events that these changes trigger as well. The depth of the outfall pipe from the proposed ballast water treatment plant will be fixed and may lie within the euphotic zone at some times of the year. It was primarily for this reason that the influence of light intensity on toxicity of oil to natural populations of phytoplankton was studied.

Valdez experiment: During April (*Acona* cruise 131) nutrient concentrations were low, (e.g., <1 µg-at NO_3^--N•liter^{-1}), and phytoplankton primary production and standing stock were very high (20 mg C•m^{-3}•hr^{-1} and ∿9 mg Chl *a*•m^{-3} respectively). Predominant forms of phytoplankton were *Monosiga marina, Phaeocystis pouchetii, Chaetoceros debilis, Fragilariopsis* sp. and *Thalassiosira nordenskiöldii* during this phase of the bloom.

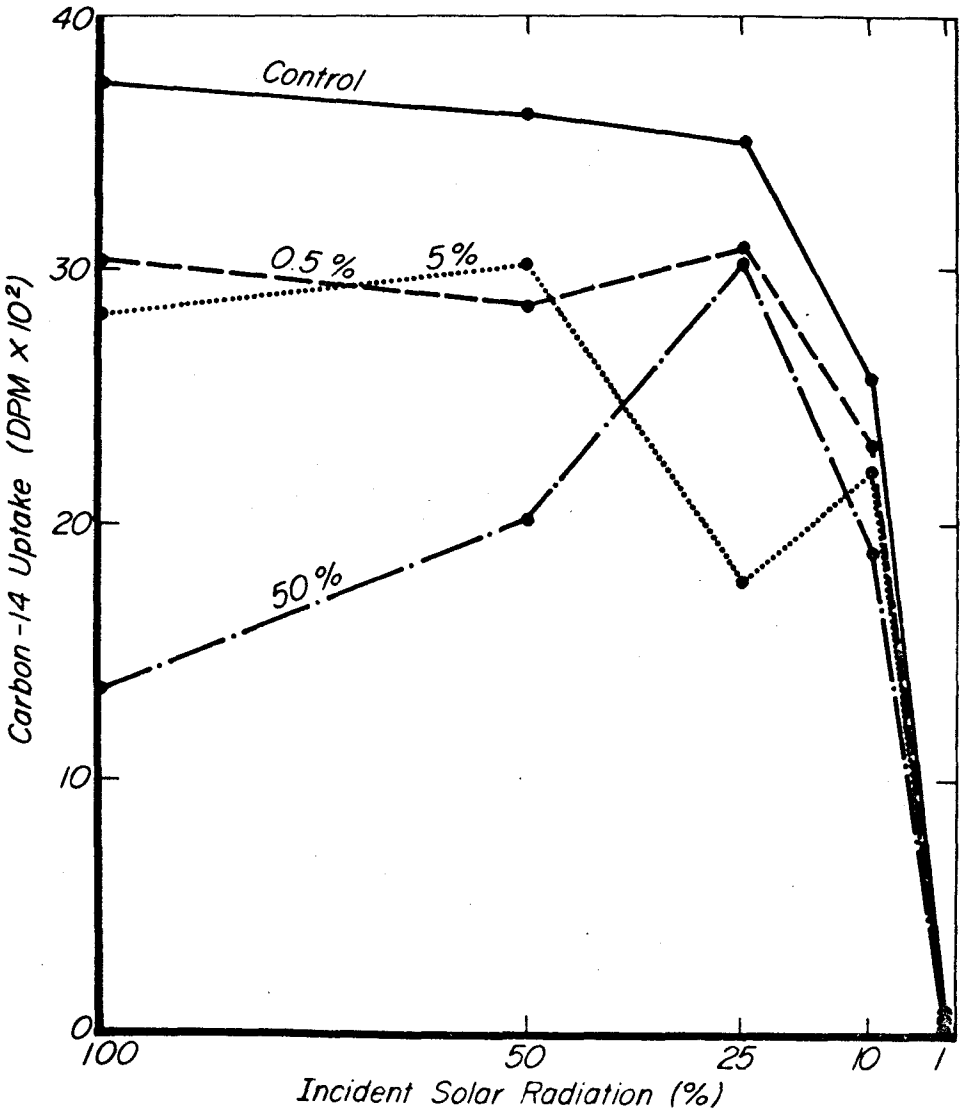
Seawater for this experiment was collected from 2.5 m near Jackson Point, mixed half and half with either an oil-seawater mixture or filtered seawater and incubated for 5 hours in a seawater-cooled incubator at 100, 50, 25, 10 and 1% of the incident surface solar

radiation (I_o) (Fig. 7). The oil concentration of the 50% oil-seawater mixture was 3.1 ppm. Maximum solar radiation during the experiment was 0.9 ly/min and averaged about 0.7 ly/min.

At 3.1 ppm crude oil a 65% reduction, and at 0.03 ppm (0.5% oil-seawater mixture) a 20% reduction in net photosynthesis was observed at full light intensity (100%). At the 25% light level and below no significant difference was noted between oil-treated and control samples.

Georgia experiment: During March 1972 a light intensity versus oil toxicity experiment, similar to that conducted in Valdez, was carried out using natural marine phytoplankton populations collected at the Skidaway Institute of Oceanography in Georgia (J. J. Goering, personal communication). Data on nutrient levels, and phytoplankton standing stock and primary production were not obtained. Fifty-milliliter bottles were filled with 40 ml raw seawater, followed by the addition of 1) 10 ml of an oil-seawater mixture (10-ml sample), 2) 5 ml oil mixture plus 5 ml filtered seawater (same used to prepare oil mixture) (5-ml sample), 3) 1 ml oil mixture plus 9 ml filtered seawater (1-ml sample), and 4) 10 ml filtered seawater (control). Each of the four samples (10-ml, 5-ml, 1-ml and control) were tested in duplicate at various light intensities (100, 50, 25, 12, 6, 3 and 0% I_o). Crude oil concentration of the 10-ml sample was about 1.6 ppm (estimated). After addition of the oil-saturated mixture the bottles were incubated for 3 hours at 19.5 C (ambient seawater temperature was 15 C) under high intensity artificial illumination at the 7 different light intensities.

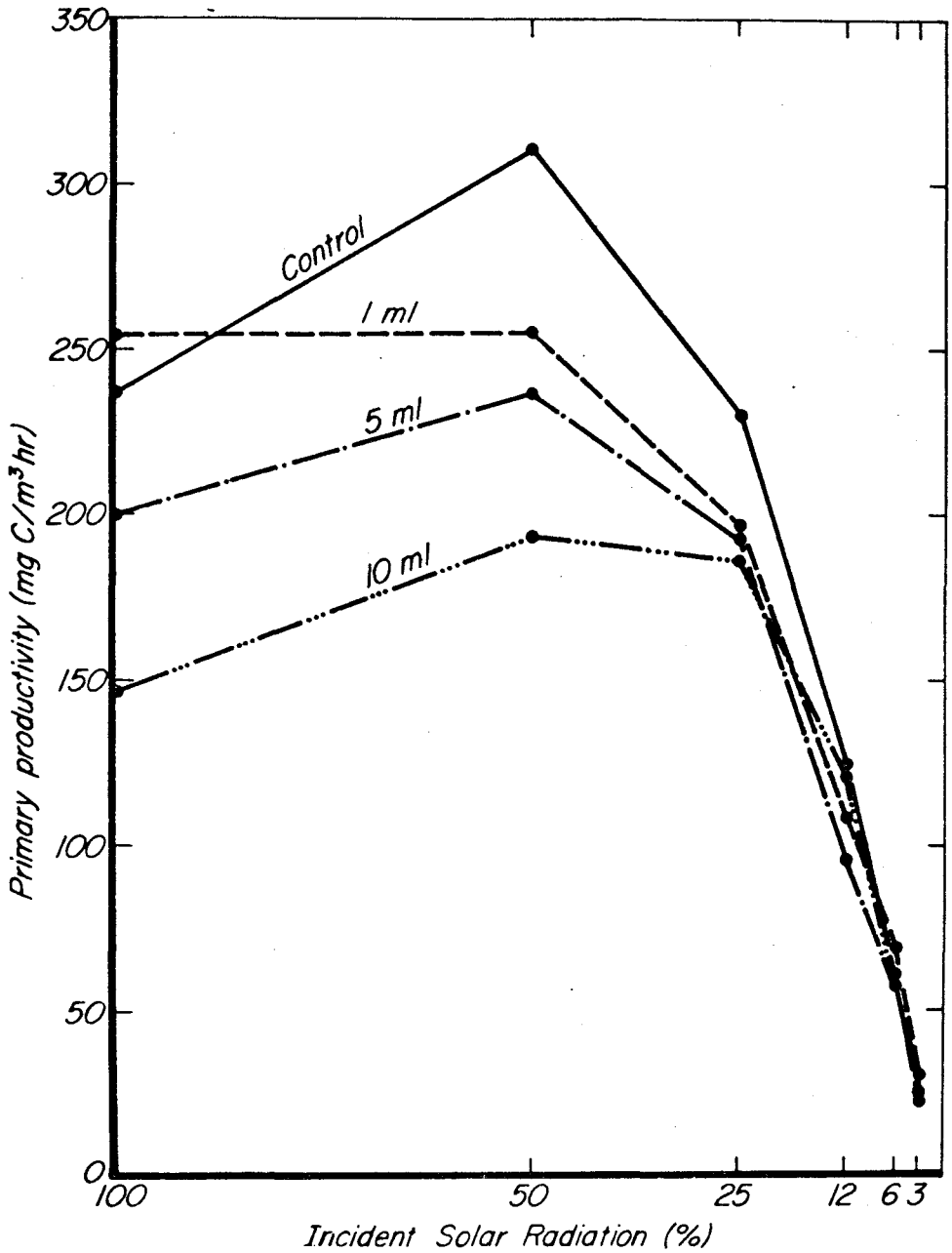
Figure 7: Photosynthesis by phytoplankton incubated for 5 hours under natural temperature conditions in relation to light intensity and to dilutions of seawater saturated with crude oil during April 1972 in Port Valdez, Alaska.


40
30
20
10
0
Control
0.5 %
5%
50%
Carbon -14 Uptake (DPM x 10²)
100
50
25
10
1
Incident Solar Radiation (%)

Each data point in figure 8 represents the mean of two samples. The control exhibited light inhibition at 100% light, in contrast to the treated samples where slight stimulation is suggested at the same intensity. At 100% light the 1-ml sample indicated a higher photosynthetic rate than the control. At the 25% light level, and below, differences between control and oil-treated samples were probably not significant.

Discussion: Photo-oxidation of crude oil may form organic acids (e.g., cyclohexane carboxylic acid) and peroxides which are known to be harmful to algae (Crafts and Reiber 1948). Either ultraviolet or sunlight may initiate this auto-oxidation, or it may occur spontaneously without light in the presence of adequate oxygen (Pilpel 1968). If photo-oxidation is proportional to light intensity, then toxicity may be likewise. The greatest differences in toxicity between control and oil-treated samples were normally noted at high light intensity (100% I_o) (Figs. 7 and 8). However, in the Georgia experiment the rate of photosynthesis was lower at 100% than at 50% I_o for the control and about the same for these light intensities in the 1-ml sample. Three processes, occurring simultaneously, may explain this observation. First, photsynthetic inhibition probably occurred as a direct result of chemical interference by crude oil. Secondly, a reduced photoinhibition caused by light absorbance by crude oil may have resulted in an *apparent* photosynthetic stimulation. And finally, the phenomenon of photosynthetic stimulation at low oil concentrations (see section 3.1.1) may have increased photosynthesis.

Figure 8: Photosynthesis by phytoplankton incubated for 3 hours under artificial conditions of light and temperature in relation to light intensity and crude oil concentration during March 1972 in Savannah, Georgia.


350
300
250
200
150
100
50
0
Primary productivity (mg C/m³hr)
Control
1 ml
5 ml
10 ml
100
50
25
12
6
3
Incident Solar Radiation (%)

Despite the differences in marine environments between Port Valdez and Savannah, Georgia, results from both experiments demonstrated similar light/oil-toxicity response patterns.

3.1.4 Exposure time and oil toxicity

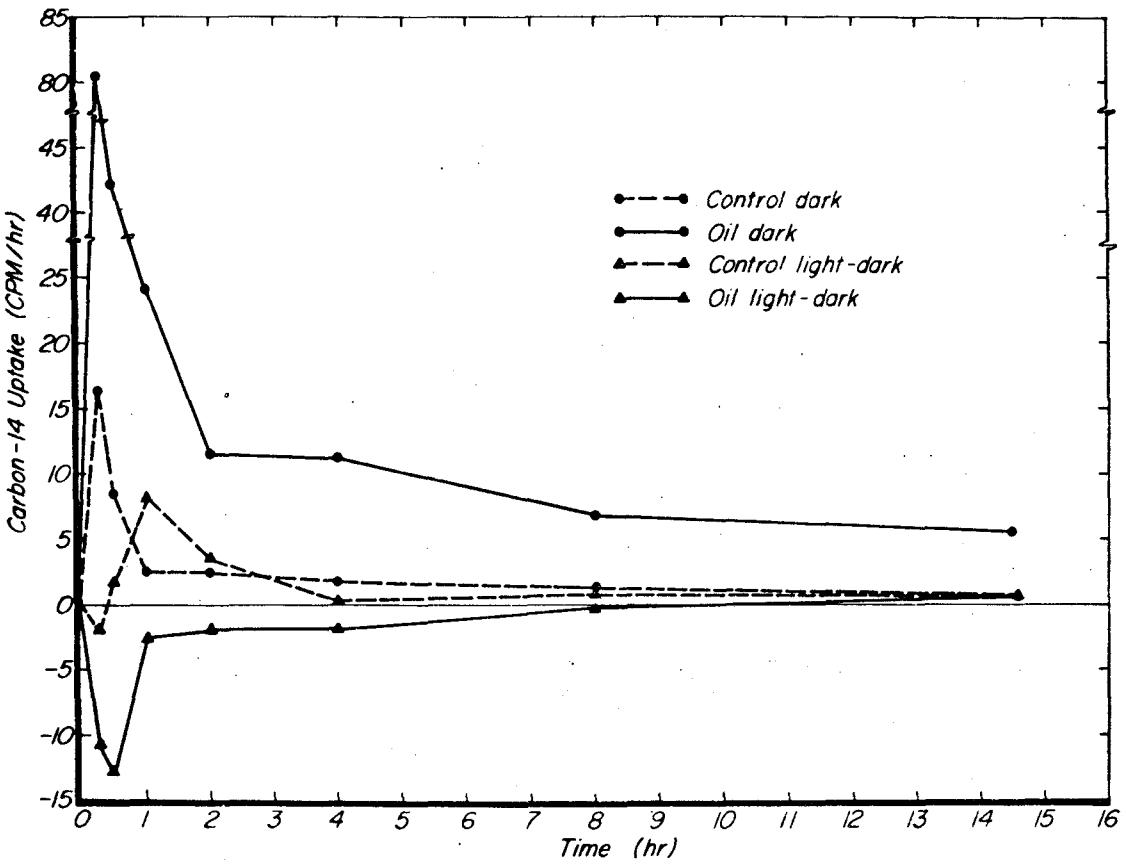
Experiments have been conducted with natural populations of phytoplankton to test the effects of prolonged exposure to oil. Incubation periods routinely used in primary productivity experiments (2 - 6 hours) may not be long enough to allow the full damage by oil to occur. Also, oil-induced changes in the species composition of a phytoplankton community may occur given sufficient time, and with routine acute toxicity tests this information is unobtainable.

December experiment: During December (*Acona* cruise 125) nutrient concentrations were moderately high (6-8 µg-at NO_3^--N·liter^{-1}), and primary production and standing stock were low (0.2 mg C·m^{-3}·hr^{-1} and 0.2 mg Chl *a*·m^{-3}). Predominant phytoplankton species were *Nitzschia closterium*, *Skeletonema costatum* and *Rhizosolenia stolterfothii*.

Seawater was collected from 2.5 m near Jackson Point by the ship's non-toxic seawater system and diluted to half with an oil-seawater mixture, or with filtered seawater. Crude oil concentration was 0.8 - 2.0 ppm (estimated) for the 10 ppm (v/v) oil-to-seawater mixture. Incubations were carried out for periods of 0.25, 0.5, 1, 2, 4, 8 and 14.5 hours under artificial light at surface seawater temperature. Light intensity during incubation simulated natural levels (0.10 ly/min).

In the controls, uptake of carbon in the dark was high during the first hour and then leveled off (Fig. 9), whereas uptake in the light

Figure 9: Photosynthesis by phytoplankton incubated under artificial light and natural seawater temperature and at a crude oil concentration of 1.3 - 3.6 ppm in relation to incubation time during December 1971 in Port Valdez, Alaska.


Control dark
Oil dark
Control light-dark
Oil light-dark
Carbon-14 Uptake (CPM/hr)
Time (hr)
85
80
45
40
25
20
15
10
5
0
-5
-10
-15
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

(represented by light-minus-dark values) indicated a high rate for the first hour and then gradually decreased. From 8 - 14.5 hours uptake appeared to be constant. In the oil-treated samples, uptake of carbon in the dark was initially very high, 300% greater than the control, and appeared to be greater than in the light until 10 hours when net photosynthesis became positive.

May experiment: During early summer (mid-May) no measurements were made for nutrients, primary production or standing stock (Chl *a*), however, cell counts indicated the standing stock to be nearly as high as was measured during April studies (Appendix A). Besides the phytoplankton species listed in figure 10, the following were also present during May: *Dictyocha fibula, Monosiga marina, Bacterosira fragilis, Coscinosira polychorda, Fragilariopsis* sp., *Skeletonema costatum, Stephanopyxis nipponica, Thalassiosira decipiens,* and *T. gravida.*

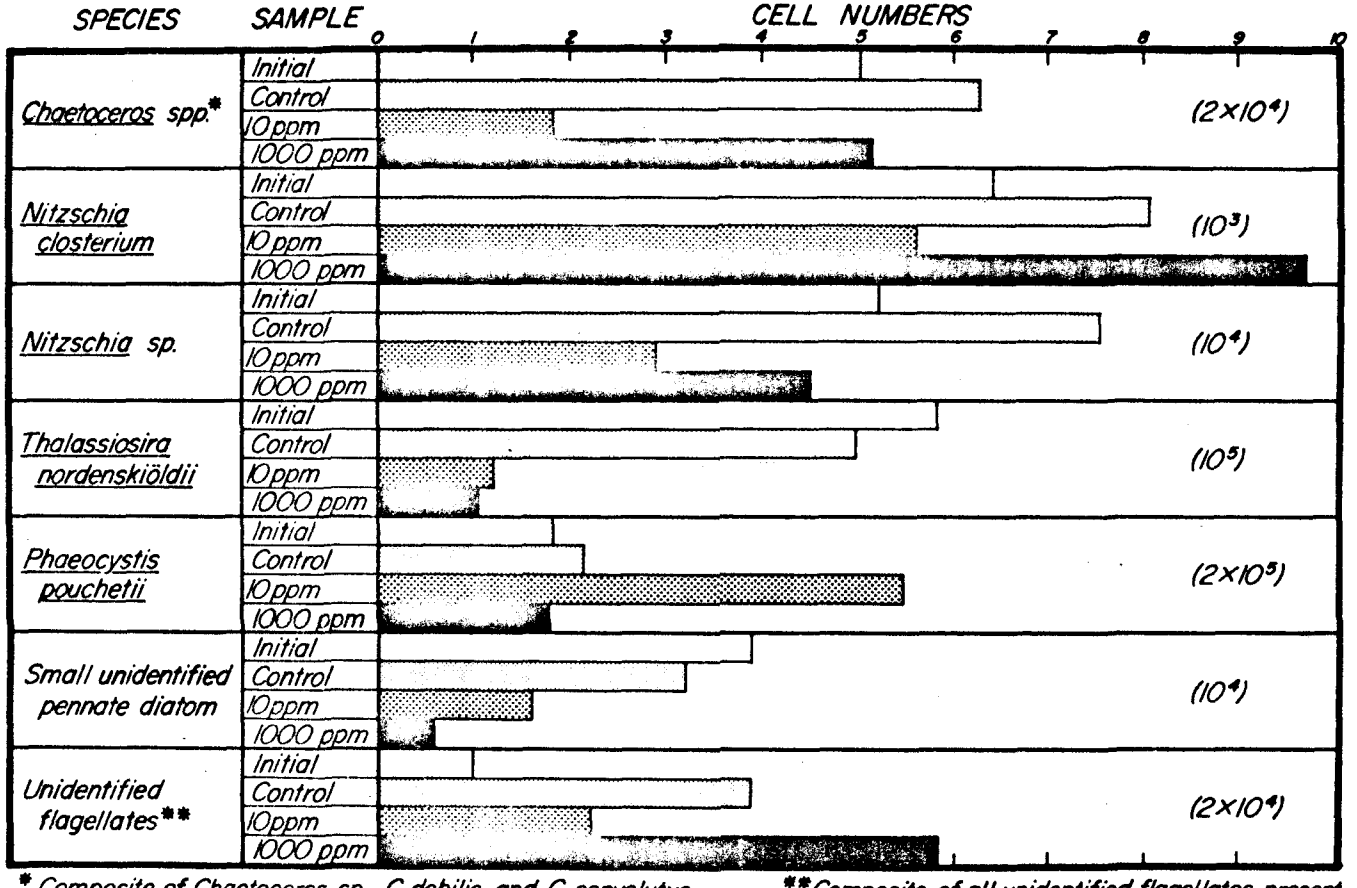
Seawater was collected from 5 m near Jackson Point and randomly dispensed into four 1-liter bottles. Contents of one bottle were immediately preserved with buffered formalin (initial), the others were incubated 48 hours under natural light and temperature conditions in a seawater-cooled incubator. Of the three bottles incubated, one contained 1 ml oil per liter (1000 ppm), another 0.01 ml per liter (10 ppm), and the third no oil (control). Following incubation the bottles were removed without agitating or disturbing the contents, and 300-ml aliquots were siphoned from the half-full level of each bottle and preserved with formalin for species identification and counting. The concentration of crude oil *in* the seawater of the incubated samples was not measured.

Errors associated with counting are considered to be small relative to sampling errors (Lund, Kipling and LeCren 1958), and are dependent upon the number of cells counted. The accuracy associated with counts for *Phaeocystis pouchetii* and *Thalassiosira nordenskiöldii* (Fig. 10) was about ±10% ($P = 0.05$), but for *Nitzschia closterium*, for example, it was as high as ±75%. Therefore, since relatively few cells were counted for *N. closterium* and the small unidentified pennate diatom, conclusions were not drawn as to oil effects on growth for these species. Counting errors of ±20% or less were associated with the remaining 5 species or groups. Sampling errors cannot be estimated since replicates were not taken; however, the interest in this experiment was in relative differences between treated and untreated samples and not in spatial and temporal differences in species abundances in the marine environment. Therefore, sampling errors were probably not serious.

Each species or species group of phytoplankton tested indicated a different growth response to crude oil at the two concentrations. The flagellates indicated greater growth rates at 1000 ppm than at 10 ppm crude oil, whereas the opposite was true for *Phaeocystis pouchetii*, which was apparently stimulated by the presence of the lower concentration of oil, showing a 150% increase in photosynthesis over the control. *Thalassiosira nordenskiöldii* was severely inhibited by both crude oil concentrations, evidenced by a 75% reduction in cell numbers.

Discussion: The high uptake rates of carbon in both oil-treated and control sample dark bottles suggest significant bacterial activity (Fig. 9). In the oil-treated samples photosynthesis after about 10 hours became positive, indicating that photosynthetic processes were still

Figure 10: Relative abundance of 7 major phytoplankton species, or species groups, in relation to additions of crude oil (v/v) in experiments incubated for 48 hours under natural conditions of light and temperature during May 1972 in Port Valdez, Alaska. Standing stocks (cells/liter) are obtained from the product of cell number times the factor given in each row.



* Composite of *Chaetoceros* sp., *C. debilis* and *C. convolutus*

** Composite of all unidentified flagellates present

functional, or that bacterial uptake was decreasing in the dark relative to the light bottles. The reason for the higher rates of carbon uptake in the dark than in the light remains unclear. Possibly light inhibition of heterotrophic growth occurred, but further studies would certainly be necessary to test this contention. Additional investigations are also needed in which both the species composition and rates of photosynthesis would be studied concurrently in crude-oil exposure-time experiments to examine possible interactions between these parameters.

Interruption in the natural species succession in a phytoplankton community is likely to have profound effects on higher food chain organisms. An understanding of how the species succession might be altered by man-made and natural influences is essential if reliable and necessary predictions of the ecological consequences of oil pollution are to be made.

Little information is available on the relative toxicities of crude oil to various phytoplankton species. Mironov and Lanskaya (1968) reported a difference in the rate of cell division between *Melosira moniliformis* and *Ditylum brightwellii* of from three to four orders of magnitude under the same conditions of exposure to kerosene, with *D. brightwellii* exhibiting the greatest sensitivity. Tests with crude oil showed similar results. Both of these species are common in Port Valdez waters (Horner *et al.*, in press).

Other important members of the phytoplankton community, such as the dinoflagellates and the yeast-like organism reported by Horner *et al.* (In press) should be investigated in a manner similar to that

used in the May experiment. The relatively high standing stocks of dinoflagellates which occurred during late summer and fall suggest their importance as members of the phytoplankton community. The yeast-like organism appeared during early spring, early summer and late summer, and often occurred in very large numbers (e.g., 1.67×10^8 cells/liter).

Several physical and chemical factors may cause changes in the species composition in phytoplankton communities: light, temperature, salinity, nutrients and dissolved substances are a few. These factors may act with oil in an adverse synergistic way, or they may act to promote vigorous growth.

3.2 Effects of Oil on Seaweeds

The near-shore area of Port Valdez, beneath which seaweeds occur, is small compared to the total surface area of the Port. The importance of this region to the entire marine ecosystem, however, is relatively large. Although the annual primary production of seaweeds is probably less than 1% of phytoplankton production in this region, seaweeds are perhaps equal in importance to the phytoplankton in their overall ecology of the system. Intertidal and subtidal seaweeds provide substrates upon which fish and invertebrate eggs may be deposited, animal habitats for a myriad of species, protective nursery areas, and in many cases the algae is used directly as a food source. The intertidal and shallow subtidal zones of Port Valdez probably have the greatest biomass as well as the greatest species diversity of any benthic community in the region.

Estimates of algal cover in the intertidal zone of Port Valdez were made during the month of August by McRoy and Stoker (1969), where in some areas they found dense stands of *Fucus distichus* covering 65% of the beach area with concurrent high animal biomass estimates, suggesting the importance of this community as an animal habitat. During late spring and summer the green algae *Cladophora, Enteromorpha, Monostroma,* and others were found to be predominant in many areas of the intertidal region at Jackson Point, with several species of red and brown algae also present (e.g., *Halosaccion, Odonthalia, Rhodymenia, Alaria* and *Fucus*). Below the intertidal zone large brown algae are found, and again these are important habitat features for many invertebrates and fishes. *Alaria marginata, Costaria costata, Desmarestia* sp., *Laminaria groenlandica* and *L. saccharina* are obvious features of this subtidal zone, and have been estimated to cover from 90 to 100 percent of the bottom to a depth of several meters in the region of Jackson Point (G. J. Mueller, personal communication).

3.2.1 Oil concentration and toxicity

Toxicity experiments were carried out to determine the effects of varying concentrations of crude oil (0.007 - 12 ppm) on photosynthesis in some predominant species of intertidal and shallow subtidal seaweeds.

July experiment: During late July (*Acona* cruise 117) nutrient content in the seawater was moderately low (e.g., NO_3^--N was ∿2 μg-at·liter^{-1}), and water temperatures ranged from 5.5 C at the surface to 11.5 C at 5 m near Jackson Point.

Specimens of *Enteromorpha intestinalis*, collected from the intertidal zone at Jackson Point were incubated in oil-saturated seawater (estimated to contain between 10 - 12 ppm crude oil), and filtered, untreated seawater for 4 hours under natural light at near-sea-surface temperatures (2.5 m). The light and dark bottle oxygen method was used to assess the damage to this species by oil.

Initial oxygen concentration was lower in the oil-seawater mixture than the filtered seawater (Fig. 11). Gross photosynthesis (light-minus-dark oxygen value) was reduced 95% when the algae were exposed to oil; net photosynthesis was zero.

October experiment: During early October (*Acona* cruise 122) nutrients were low (NO_3^--N was ∿1 μg-at·liter^{-1}) and surface water temperatures ranged from 6.5 - 9.5 C.

Specimens of *Fucus distichus*, collected from the intertidal zone at Jackson Point, were incubated in seawater containing various concentrations of crude oil under the same conditions as described for the July *Enteromorpha intestinalis* experiment.

Dissolved oxygen concentration in initial bottles was inversely proportional to oil concentration (Fig. 12). Gross photosynthesis showed relatively little change over the concentration range of oil tested. The highest rate of photosynthesis appeared at the 100% mixture (10 - 12 ppm), with the lowest rate noted for an intermediate oil concentration (25%).

April experiment: During late April (*Acona* cruise 131) the nutrient content was very low with NO_3^--N <1 μg-at·liter^{-1}. Seawater temperature ranged from 2 - 3 C in the euphotic zone.

Figure 11: Metabolism of the green seaweed *Enteromorpha intestinalis* incubated for 4 hours under natural conditions of light and temperature in relation to seawater saturated with crude oil as measured by the light and dark bottle oxygen method during July 1971 in Port Valdez, Alaska.

SAMPLE	Initial O_2	Final O_2		Dry Weight (gm)	
		Lt.	Dk.	Lt.	Dk.
Seawater (S.W.)	6.27	6.44	5.47		
Algae + S.W.	6.27	12.01	4.64	.3116	.3850
S.W. / Oil	4.57	5.72	4.58		
Algae + S.W. / Oil	4.57	5.12	3.51	.2783	.3284

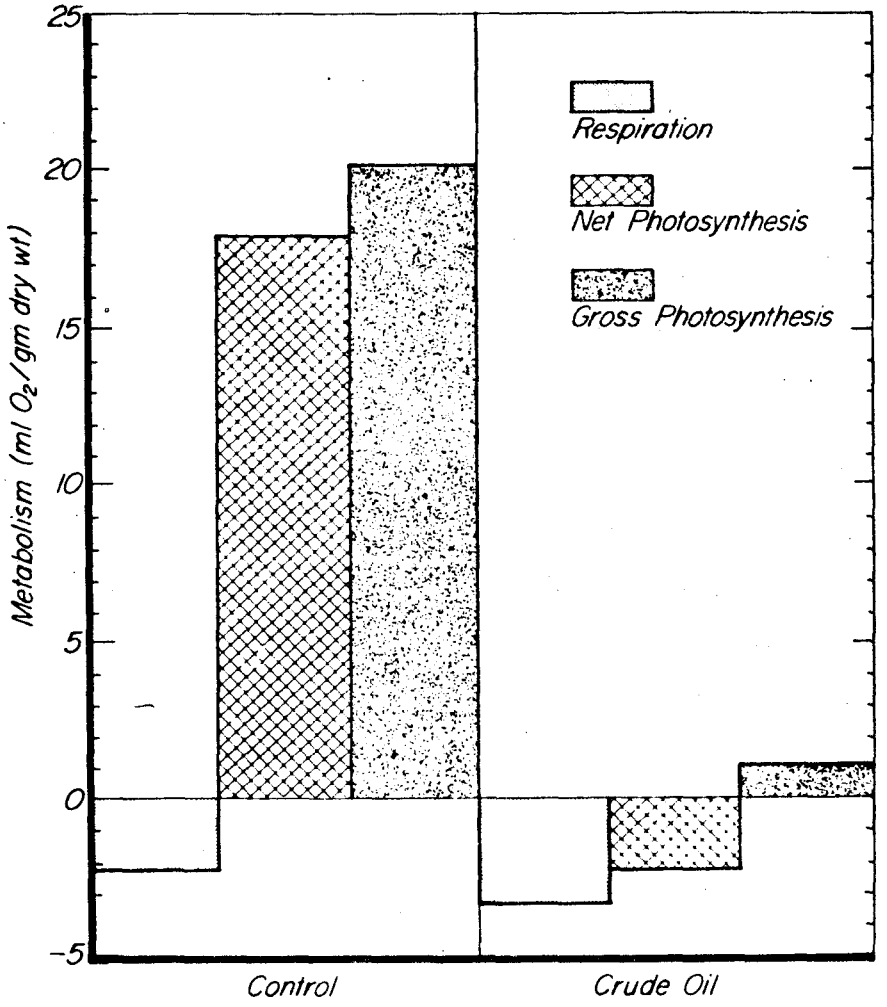
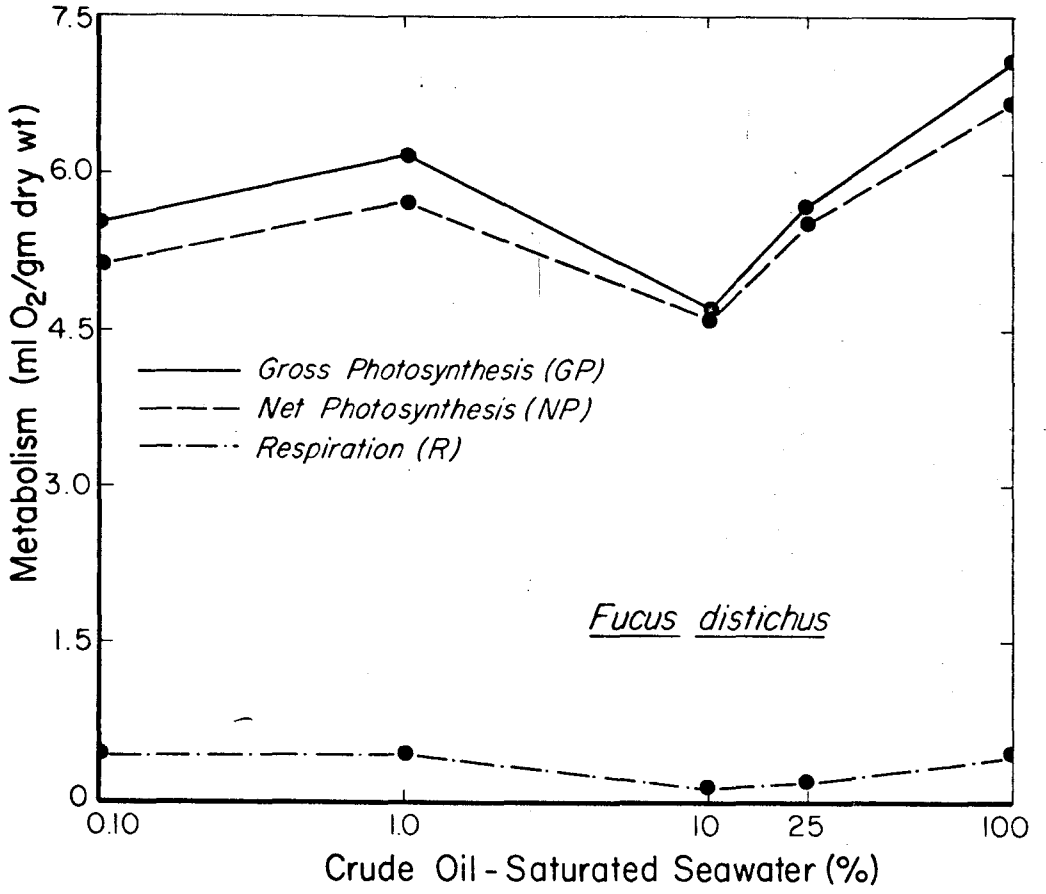


Figure 12: Metabolism of the brown seaweed *Fucus distichus* incubated for 4 hours under natural conditions of light and temperature in relation to dilutions of seawater saturated with crude oil as measured by the light and dark bottle oxygen method during October 1971 in Port Valdez, Alaska.

CRUDE OIL SATURATION	DISSOLVED OXYGEN (ml/l)			PLANT DRY WEIGHT (gm)		METABOLISM		
	IB	LB	DB	LB	DB	GP	NP	R
100%	3.22	16.65	2.21	2.01	2.35	7.11	6.68	0.43
25%	5.09	20.76	4.63	2.83	2.68	5.71	5.54	0.17
10%	5.30	22.33	5.06	3.65	4.01	4.73	4.67	0.06
1%	5.43	17.21	3.65	2.05	3.99	6.19	5.75	0.45
0.1%	5.59	17.66	4.60	2.36	2.40	5.53	5.11	0.41



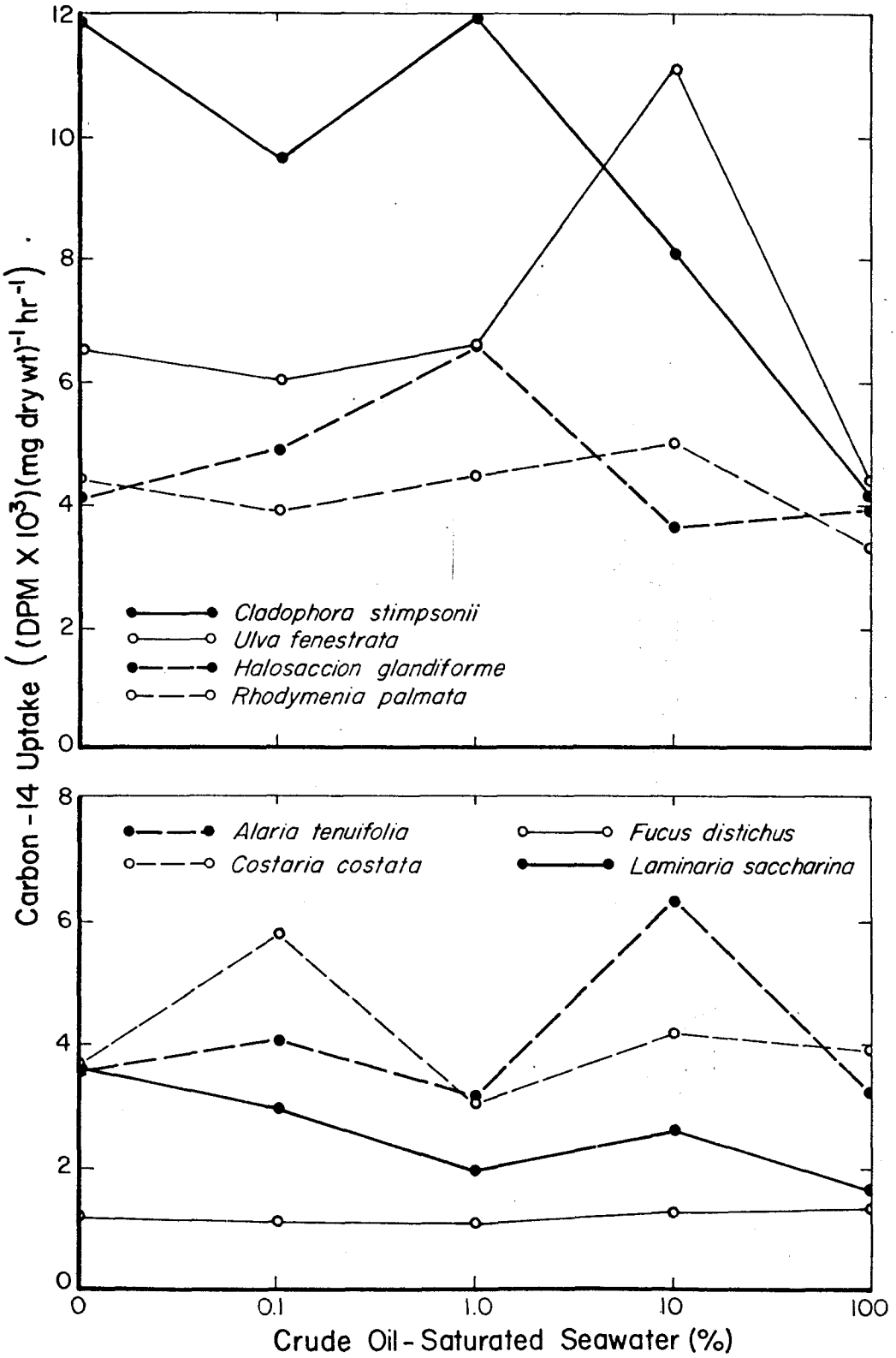
The ^{14}C method was used to test 8 species of seaweeds for toxicity of crude oil. The plants were incubated for 2 - 4 hours in filtered seawater containing various concentrations of crude oil under natural conditions of temperature and light in a seawater-cooled incubator. Oil concentration of the 100% oil-seawater mixture was 7 ppm.

It is uncertain how representative the curves shown in figure 13 are to the species tested, since only healthy specimens were selected for testing and replicates were not taken. The systematic errors, however, may be estimated by assuming that the standard deviation for five subsamples of *Rhodymenia palmata* (incubated in the light at 0.007 ppm crude oil) is representative for all species at all oil concentrations. The 95% confidence limits calculated for the mean and applied to the other plant species is ±4%.

Photosynthetic response to oil-treated seawater was different for each species; some showed inhibition at 7 ppm while others appeared unaffected. *Cladophora stimpsonii* indicated a 65% photosynthetic reduction at 7 ppm while *Costaria costata* appeared to be unaffected at the same concentration. Photosynthetic stimulation by crude oil was suggested for some species; at 0.7 ppm *Ulva fenestrata* had a rate nearly twice the control.

Discussion: Literature on the effects of petroleum products on seaweeds is primarily from studies of economically important species. In tests with the giant brown alga, *Macrocystis pyrifera*, Clendenning (1959) found that diesel oil, emulsified in seawater to give a 1% by volume oil concentration, reduced photosynthetic capacity 25% after

Figure 13: Photosynthesis by 8 species of seaweeds incubated for 2 - 4 hours under natural conditions of light and temperature in relation to dilutions of seawater saturated with crude oil during May or April 1972 in Port Valdez, Alaska. Actural DPM values for *Alaria tenuifolia* are twice those indicated.


Carbon-14 Uptake ((DPM X 10³)(mg dry wt)⁻¹ hr⁻¹)
12
10
8
6
4
2
0
Cladophora stimpsonii
Ulva fenestrata
Halosaccion glandiformis
Rhodymenia palmata
8
6
4
2
0
Alaria tenuifolia
Costaria costata
Fucus distichus
Laminaria saccharina
0
0.1
1.0
10
100
Crude Oil-Saturated Seawater (%)

24 hours and 100% after 72 hours of exposure. If the kelp were exposed to a diesel oil emulsion for a period of 6 hours, irreversible damage was done, presumably because at least that much time was required for penetration of the oil into the cytoplasmic membrane (Van Overbeek and Blondeau, 1954). Since no experiments in this study were conducted for periods that long, it is quite likely that the total extent of damage was not observed.

The green alga *Enteromorpha intestinalis* is considered a very hardy species, being both eurythermal and euryhaline (Biebl 1962). However, gross photosynthesis was greatly reduced when the plant was exposed to 10 ppm crude oil for 4 hours (Fig. 11).

The biochemical oxygen demand (B.O.D.) was observed to be higher in oil-treated than control seawater in both experiments employing the oxygen method (Figs. 11 and 12). Since the seawater used to prepare the oil-seawater mixture was Millipore^{R}-filtered and pasteurized, and the crude oil that was used is thought to be nearly sterile (D. K. Button, personal communication), auto- rather than bio-oxidation probably accounted for the higher B.O.D.

Rates of photosynthesis determined by the oxygen and ^{14}C methods cannot be reliably compared since the photosynthetic quotient must be known in the oxygen method and this value depends upon the physiological state of the organism and the immediate environmental conditions. For example, with the rate of carbon dioxide assimilation constant, the amount of oxygen evolved by algae may vary whether nitrate or ammonia serves as the nitrogen source with higher oxygen evolution resulting from nitrate utilization (Strickland 1960).

In the ^{14}C experiments, each species of algae appeared to respond differently to the various crude oil concentrations. Crude oil inhibition of photosynthesis by the green alga *Cladophora stimpsonii* was higher than other species tested (∿60% at 7 ppm). The higher surface to volume ratio of this species, and the absence of the thick mucilaginous covering typical of other algae may explain these results. The mucilage associated with many algal species is known to protect them from petroleum substances over short exposure periods (Clendenning 1959; Schramm 1971).

Limitations of the seaweed toxicity experiments conducted in this study were the length of exposure time (2 - 4 hours), and the absence of information on the toxic effects of oil to reproductive forms of the algae (gametes, zoospores, etc.). Since the protective covering of mucilage typical of mature plants is often absent in reproductive forms, severe damage to a seaweed population could occur, for example, if seawater were polluted by oil during the time of year gametes are released.

4. SUMMARY

The effects of crude oil as a pollutant in the Port Valdez marine environment were investigated with respect to oil toxicity to photosynthesis by indigenous populations of phytoplankton and important seaweed species. Toxicity experiments were conducted during various times of the year and thus under differing environmental conditions. A summary of experimental results is given:

1. The concentrations of crude oil in seawater necessary to cause a specific degree of photosynthetic inhibition apparently change seasonally depending on physical and chemical factors and on the species composition and relative abundances. During June a 50% inhibition to phytoplankton photosynthesis occurred at approximately 2.0 ppm crude oil.

2. Crude oil in very low concentrations in seawater stimulated phytoplankton photosynthesis over short incubation periods. This phenomenon occurred during December, April and June. The photosynthetic rate of June phytoplankton exposed to a concentration of about 0.003 ppm crude oil was more than double the rate for phytoplankton in seawater containing no oil.

3. Crude oil in treated ballast water appeared to be about one-tenth as inhibitory to photosynthesis as fresh crude oil when tested on phytoplankton collected in June.

4. The effect of temperature on oil toxicity to phytoplankton gave varied results. The temperature optimum for photosynthesis differed between experiments. Phytoplankton treated with crude oil showed maximum photosynthesis (light values) at 5 C during March, 10 - 15 C during April, and 20 C during August at crude oil concentrations between 1 - 4 ppm. The patterns for the oil-contaminated samples did not follow that of the controls. During March the oil-treated samples showed decreased photosynthesis with increasing temperature; controls indicated an optimum temperature for photosynthesis at about 10 C. In contrast, during April the oil-treated samples showed a maximum rate of photosynthesis at 10 - 15 C compared to a 10 C optimum for controls.

5. The rate of light intensity in oil toxicity to phytoplankton was examined. At high natural light levels crude oil toxicity appeared to be acute. Phytoplankton exposed to about 5.5 ppm showed a 65% reduction in photosynthesis at full light intensity (maximum of 0.89 ly/min), although phytoplankton exposed to the same concentration of crude oil at one-fourth this intensity showed only a 20% reduction. A similar pattern was observed during tests with phytoplankton from a contrasting marine environment (Savannah, Georgia) subjected to the same crude oil.

6. The relative species composition in a natural phytoplankton population may be altered by crude oil, resulting in a decrease in the abundance of some species and an increase in others. This effect is apparently dependent upon the amount of oil present.

During May *Phaeocystis pouchetii* cells more than doubled in number after a 48-hour exposure to a crude oil concentration of 10 ppm (0.01 ml oil/added liter). In contrast, the number of *Thalassiosira nordenskiöldii* cells, was reduced about 75%.

7. Seaweed oil-toxicity experiments, in which the ^{14}C method was used to measure photosynthesis, indicated no pattern that was common to all species. Photosynthetic inhibition was indicated for *Cladophora stimpsonii*, *Ulva fenestrata* and *Laminaria saccharina* at 7 ppm crude oil, whereas other species were not significantly affected at this concentration. Photosynthetic stimulation was noted for several algae at different concentrations (∿80% photosynthetic increase by *Ulva* and *Alaria* at 0.7 ppm crude oil; ∿30% increase by *Costaria* at 0.007 ppm). *Enteromorpha intestinalis* showed an 80% reduction in oxygen production when exposed to 10 - 12 ppm oil.

The marine plant populations in Port Valdez, Alaska, showed varied photosynthetic responses to crude oil contamination. These responses seemed to result from a complex interaction of several physical, chemical and biological factors that can apparently act in either an adverse or beneficial way.

A greater understanding of these interactions within this system is essential before reliable predictions of the total effects of crude oil introduction into this marine environment can be made.

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APPENDICES

A - D

Explanation

The data presented in Appendices A through C were collected at approximately the same times (*Acona* cruises 117, 122, 125, 128 and 131) and location (station 153, Jackson Point) as were seawater samples used in oil toxicity experiments conducted between August 1971 and April 1972.

Appendix A

Phytoplankton Standing Stocks

(cells/liter)

May 1971 to April 1972[1]

[1]from Horner *et al.* (In press)

Explanation

The dates and locations of sampling are given on following summary sheets as cruise number (eg. *Acona* 117-153) and station number (eg. *Acona* 117-153).

Cruise	Date
Acona 113	16 to 26 May 1971
Acona 117	27 July to 4 Aug. 1971
Acona 122	4 to 13 Oct. 1971
Acona 125	29 Nov. to 8 Dec. 1971
Acona 128	9 to 20 Mar. 1972
Acona 131	21 to 30 Apr. 1972

ACONA 117-153 100% 5ml 40X

Yeast-like organisms-too many to count; at least 10,000 in one transect across counting chamber.

ACONA 117-153	1%	5ml	40X	4.8 CELLS/LITER
DIATOMS				CELLS/LITER
Unidentified diatoms				1.2
Unidentified Organisms				
8 µm				0.4
15 µm				3.2

ACONA 122-153 100% 5ml 40X 144.2 CELLS/LITER

DIATOMS	CELLS/LITER
Amphora sp.	0.4
Chaetoceros sp.	0.8
Leptocylindrus danicus	0.4
Nitzschia closterium	25.2
Nitzschia paradoxa	0.8
Rhizosolenia stolterfothii	3.6
Skeletonema costatum	4.8
Thalassiosira gravida	3.2
Unidentified diatoms 	8.8
FLAGELLATES	
Monosiga marina cf.	0.4
Unidentified flagellates	
2 µm	41.6
3 µm	53.8
6 µm	12.2
6 µm	10.8
10 µm	6.8
Unidentified organisms	6.4

ACONA 122-153 50% 3m 5ml 40X 101.2 CELLS/LITER

DIATOMS	CELLS/LITER
Chaetoceros sp.	0.4
Leptocylindrus danicus	8.0
Nitzschia closterium	8.4
Nitzschia seriata	1.6
Nitzschia sp.	1.2
Rhizosolenia stolerfothii	4.4
Skeletonema costatum	9.2
Thalassiosira gravida	2.4
FLAGELLATES	
Monosiga marina cf.	0.8
Dictyocha fibula	1.2
Unidentified flagellates	
2 µm 	8.0
3 µm 	10.4
3 µm 	2.8
5 µm 	7.6
5 µm 	0.8
5 µm 	20.8
9 µm 	0.8
Unidentified organisms 	12.4

ACONA 122-153 25% 7.5m 5ml 40X 78.2 CELLS/LITER

DIATOMS	CELLS/LITER
Cocconeis sp.	0.4
Coscinodiscus oculus iridis	0.4
Leptocylindrus danicus	1.6
Navicula sp.	0.8
Nitzschia closterium	9.6
Nitzschia sp.	0.8
Skeletonema costatum	1.6
Thalassiosira gravida	1.2
Unidentified diatoms 	0.8
FLAGELLATES	
Dictyocha fibula	1.2
Unidentified flagellates	
3 µm 	12.8
3-4 µm 	2.8
4 µm 	7.2
5 µm 	18.2
8 µm 	3.6
12 µm 	7.2
Unidentified organisms 	8.4

ACONA 122-153 10% 12.5m 5ml 40X 58.8 CELLS/LITER

DIATOMS	CELLS/LITER
Coscinodiscus radiatus	0.4
Coscinodiscus sp.	0.4
Leptocylindrus danicus	9.6
Nitzschia sp.	0.4
Skeletonema costatum	1.6
Thalassiosira gravida cf.	9.2
Thalassiosira sp.	0.8
Unidentified diatoms 	1.6
FLAGELLATES	
Unidentified flagellates	
5 µm 	3.6
12 µm 	1.2
Unidentified organisms	30.0

ACONA 122-153 1% 5ml 40X 33.6 CELLS/LITER

DIATOMS	CELLS/LITER
Chaetoceros sp.	3.6
Leptocylindrus danicus	4.4
Nitzschia closterium	1.6
Nitzschia sp.	1.2
Thalassionema nitzschioides	1.2
Thalassiosira nordenskiöldii	3.2
FLAGELLATES	
Unidentified flagellates	
3 µm	14.4
14 µm	1.2
DINOFLAGELLATES	
Dinophysis rotundatum	0.4
Unidentified organisms	1.2

ACONA 125-153 100% 5ml 40X 14.0 CELLS/LITER

DIATOMS	CELLS/LITER
Coscinodiscus sp.	0.4
Nitzschia closterium	0.4
Unidentified diatoms 	1.2

FLAGELLATES

Unidentified flagellates	
3 µm *o*	4.0
6 µm *O*	0.8
8 µm *o*	0.4
8 µm *O*	6.8

ACONA 125-153 50% 5ml 40X 9.2 CELLS/LITER

DIATOMS CELLS/LITER

None

FLAGELLATES

Unidentified flagellates

2 µm 0 0.4

3 µm ⊙ 8.0

4 µm 0 0.8

ACONA 125-153 25% 5ml 40X 14.8 CELLS/LITER

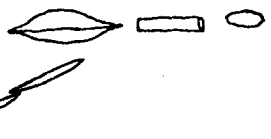
DIATOMS	CELLS/LITER
Chaetoceros sp.	0.4
Thalassiosira sp.	0.4

FLAGELLATES

Unidentified flagellates

3 µm	9.2
4 µm	2.8
6 µm	1.6
----	0.8

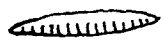
ACONA 125-153 10% 5ml 40X 29.6 CELLS/LITER

DIATOMS	CELLS/LITER
Coscinodiscus radiatus	0.4
Nitzschia closterium	2.0
Nitzschia sp.	2.0
Rhizosolenia stolterfothii	0.4
Skeletonema costatum	0.8
Thalassiosira sp.	0.4
Unidentified diatoms 	2.0

FLAGELLATES

Unidentified flagellates	
2 µm 	9.6
3 µm 	4.4
4 µm 	7.2
---- 	0.4
---- 	0.4

ACONA 125-153 1% 5ml 40X 22.4 CELLS/LITER

DIATOMS	CELLS/LITER
Nitzschia closterium	0.8
Rhizosolenia stolterfothii	0.4
Skeletonema costatum	1.6
Thalassiosira gravida	0.4
Unidentified diatoms 	0.4

FLAGELLATES

Unidentified flagellates	
2 µm	8.0
3 µm 	1.6
5 µm 	7.2
---- 	0.4
5 µm 	0.8
7 µm 	0.4

ACONA 128-153 100% 5ml 40X 8.8 CELLS/LITER

DIATOMS	CELLS/LITER
Nitzschia closterium	1.6
Thalassionema nitzschioides	0.8

FLAGELLATES

Unidentified flagellates	
2 µm 0	5.6
5 µm 0	0.8

ACONA 128-153	50%	5ml	40X	7,160.4 CELLS/LITER
DIATOMS				CELLS/LITER
Nitzschia closterium				0.4
Unknown organisms				
cf yeast				7,160.0

ACONA 128-153 25% 5ml 40X 41.2 CELLS/LITER

DIATOMS CELLS/LITER

None

FLAGELLATES

Unknown flagellates

3 µm 0	33.2
5 µm 0	0.4
6 µm 0	6.8
8 µm 0	0.8

ACONA 128-153 10% 5ml 40X 3.2 CELLS/LITER

DIATOM	CELLS/LITER
Nitzschia closterium	0.8
Unidentified diatoms ▭	0.4
FLAGELLATES	
Unknown flagellates	
2 µm O	2.0

ACONA 128-153 1% 5ml 40X 374.8 CELLS/LITER

DIATOMS CELLS/LITER

None

FLAGELLATES

Unknown flagellates

2 µm 369.6

3 µm 0.8

4 µm 0.8

Unidentified organisms 3.6

ACONA 131-153 100% 5ml 40X 4156.8 CELLS/LITER

DIATOMS	CELLS/LITER
Bacterosira fragilis	49.6
Chaetoceros brevis	5.6
Chaetoceros debilis	105.6
Chaetoceros subsecundus	3.2
Fragilariopsis sp.	24.8
Nitzschia closterium	9.6
Nitzschia pungens	1.6
Nitzschia sp.	5.6
Skeletonema costatum	16.8
Thalassionema nitzschioides	1.6
Thalassiosira decipiens	48.0
Thalassiosira gravida	16.8
Thalassiosira nordenskiöldii	184.0
Unidentified diatoms 	0.8
FLAGELLATES	
Monosiga marina cf.	828.8
Phaeocystis pouchetii	2791.2
Unidentified flagellates	
3 µm 	6.4
DINOFLAGELLATES	
Peridinium minusculum	5.6

ACONA 131-153 50% 5ml 40X 2569.9 CELLS/LITER

DIATOMS	CELLS/LITER
Bacterosira fragilis	11.2
Chaetoceros atlanticus	3.2
Chaetoceros debilis	208.0
Coscinosira (Thalassiosira) polychorda	7.2
Fragilariopsis sp.	124.0
Nitzschia closterium	4.8
Nitzschia sp.	26.4
Skeletonema costatum	21.6
Thalassiosira decipiens	28.8
Thalassiosira gravida	8.8
Thalassiosira nordenskiöldii	172.8
Unidentified diatoms	2.4
FLAGELLATES	
Monosiga marina cf.	39.6
Phaeocystis pouchetii	1906.4
Unidentified flagellates	
3 µm	0.8
3 µm	1.6

ACONA 131-153 25% 5ml 40X 1747.2 CELLS/LITER

DIATOMS	CELLS/LITER
Bacterosira fragilis	12.8
Chaetoceros debilis	124.8
Coscinosira (Thalassiosira) polychorda	4.8
Fragilariopsis sp.	128.8
Nitzschia closterium	4.0
Nitzschia pungens	2.4
Nitzschia sp.	22.4
Skeletonema costatum	11.2
Thalassiosira decipiens	12.8
Thalassiosira nordenskiöldii	73.6
Unidentified diatoms 	3.2

FLAGELLATES	
Monosiga marina cf.	259.2
Dictyocha fibula	0.8
Phaeocystis pouchetii	1076.0
Unidentified flagellates 0 ----	9.6

ACONA 131-153 1% 5ml 40X 2506.4 CELLS/LITER

DIATOMS	CELLS/LITER
Bacterosira fragilis	23.2
Biddulphia aurita	2.4
Chaetoceros debilis	128.8
Coscinosira (Thalassiosira) polychorda	4.0
Fragilariopsis sp.	180.0
Nitzschia closteriumum	10.4
Nitzschia pungens	2.4
Nitzschia sp.	0.8
Thalassiosira nitzschioides	2.4
Thalassiosira decipiens	36.6
Thalassiosira gravida	52.8
Thalassiosira nordenskiöldii	92.8
Unidentified diatoms	0.8
FLAGELLATES	
Monosiga marina cf.	448.0
Phaeocystis pouchetii	1488.8
DINOFLAGELLATES	
Peridinium minusculum	0.8

Appendix B

Species Lists of Phytoplankton Collected in Vertical Tows, May 1971 to April 1972[1]

[1] from Horner *et al.* (In press)

Explanation

The dates and locations of sampling are given on the following summary sheets as cruise number (eg. *Acona* 117-153) and station number (eg. *Acona* 117-153).

Cruise	Date
Acona 113	16 to 26 May 1971
Acona 117	27 July to 4 Aug. 1971
Acona 122	4 to 13 Oct. 1971
Acona 125	29 Nov. to 8 Dec. 1971
Acona 128	9 to 20 Mar. 1972
Acona 131	21 to 30 Apr. 1972

ACONA 113-153, vertical tow

DIATOMS

Biddulphia aurita

Chaetoceros decipiens

Coscinodiscus centralis
Coscinodiscus oculus iridis

Fragilariopsis sp.

Nitzschia sp.

DINOFLAGELLATES

Dinophysis sp.

Peridinium conicum

OTHER PHYTOPLANKTON

Pterosperma sp.

ZOOPLANKTON

Copepods

ACONA 117-153, 20 m to surface, vertical tow

DIATOMS

Chaetoceros decipiens

Melosira sp. cf. *moniliformis*

DINOFLAGELLATES

Ceratium fusus
Ceratium lineatum
Ceratium tripos

OTHER PHYTOPLANKTON

ZOOPLANKTON

Copepods, nauplii
Tintinnids

ACONA 122-153, 35 m, vertical tow

DIATOMS

Chaetoceros compressus
Chaetoceros convolutus
Chaetoceros decipiens
Chaetoceros radicans
Chaetoceros sp.

Coscinodiscus curvatulus
Coscinodiscus excentricus

Ditylum brightwellii

Nitzschia seriata

Skeletonema costatum

Thalassionema nitzschioides

Thalassiosira spp.

DINOFLAGELLATES

Ceratium fusus
Ceratium longipes
Ceratium tripos

Peridinium conicum
Peridinium depressum
Peridinium pallidum

OTHER PHYTOPLANKTON

Dictyocha fibula

ZOOPLANKTON

Foraminiferans
Radiolarians

ACONA 125-153, vertical tow

DIATOMS

Biddulphia aurita

Chaetoceros atlanticus
Chaetoceros brevis
Chaetoceros concavicornis
Chaetoceros debilis
Chaetoceros decipiens

Coscinodiscus centralis
Coscinodiscus radiatus

Ditylum brightwellii

Gyro-Pleurosigma sp.

Leptocylindrus danicus

Melosira moniliformis

Nitzschia pungens
Nitzschia seriata
Nitzschia sp.

Rhizosolenia stolterfothii
Rhizosolenia styliformis

Skeletonema costatum

Stephanopyxis nipponica

Thalassiosira nordenskioldii

Unidentified pennates

DINOFLAGELLATES

Ceratium fusus
Ceratium lineatum
Ceratium longipes
Ceratium tripos

Peridinium depressum
Peridinium pallidum

OTHER PHYTOPLANKTON

Dictyocha fibula

ZOOPLANKTON

ACONA 128-153, 29.5m, vertical tow

DIATOMS

Biddulphia aurita
Biddulphia sp. (cf. *striata*)

Chaetoceros atlanticus
Chaetoceros convolutus
Chaetoceros decipiens

Coscinodiscus centralis
Coscinodiscus oculus iridis

Ditylum brightwellii

Fragilariopsis sp.

Grammatophora marina

Gyro-Pleurosigma sp.

Melosira moniliformis
Melosira sulcata

Nitzschia closterium
Nitzschia bilobata

Phizosolenia alata

Thalassionema nitzschioides

Thalassiosira nordenskiöldii

Triceratium arcticum

DINOFLAGELLATES

Ceratium fusus
Ceratium longipes

Peridinium depressum
Peridinium pallidum

ZOOPLANKTON

Copepods

ACONA 131-153, 15m, vertical tow

DIATOMS

Bacterosira fragilis

Biddulphia aurita

Chaetoceros convolutus
Chaetoceros debilis
Chaetoceros decipiens

Coscinodiscus oculus iridis

Coscinosira (Thalassiosira) polychorda

Fragilariopsis sp.

Leptocylindrus danicus

Nitzschia closterium
Nitzschia seriata

Rhizosolenia hebatata

Stephanopyxis nipponica

Thalassiosira decipiens
Thalassiosira gravida
Thalassiosira nordenskiöldii

DINOFLAGELLATES

OTHER PHYTOPLANKTON

Phaeocystis pouchetii

ZOOPLANKTON

Copepods

Appendix C

Nutrient[1], Dissolved Oxygen[2] and Total

Carbon Dioxide[3] Profiles and Phytoplankton

Stanking Stock[4] and Primary Productivity[5] Data

May 1971 to April 1972

[1] from Goering, Patton and Shiels (In press)

[2,3] from Hood and Patton (In press)

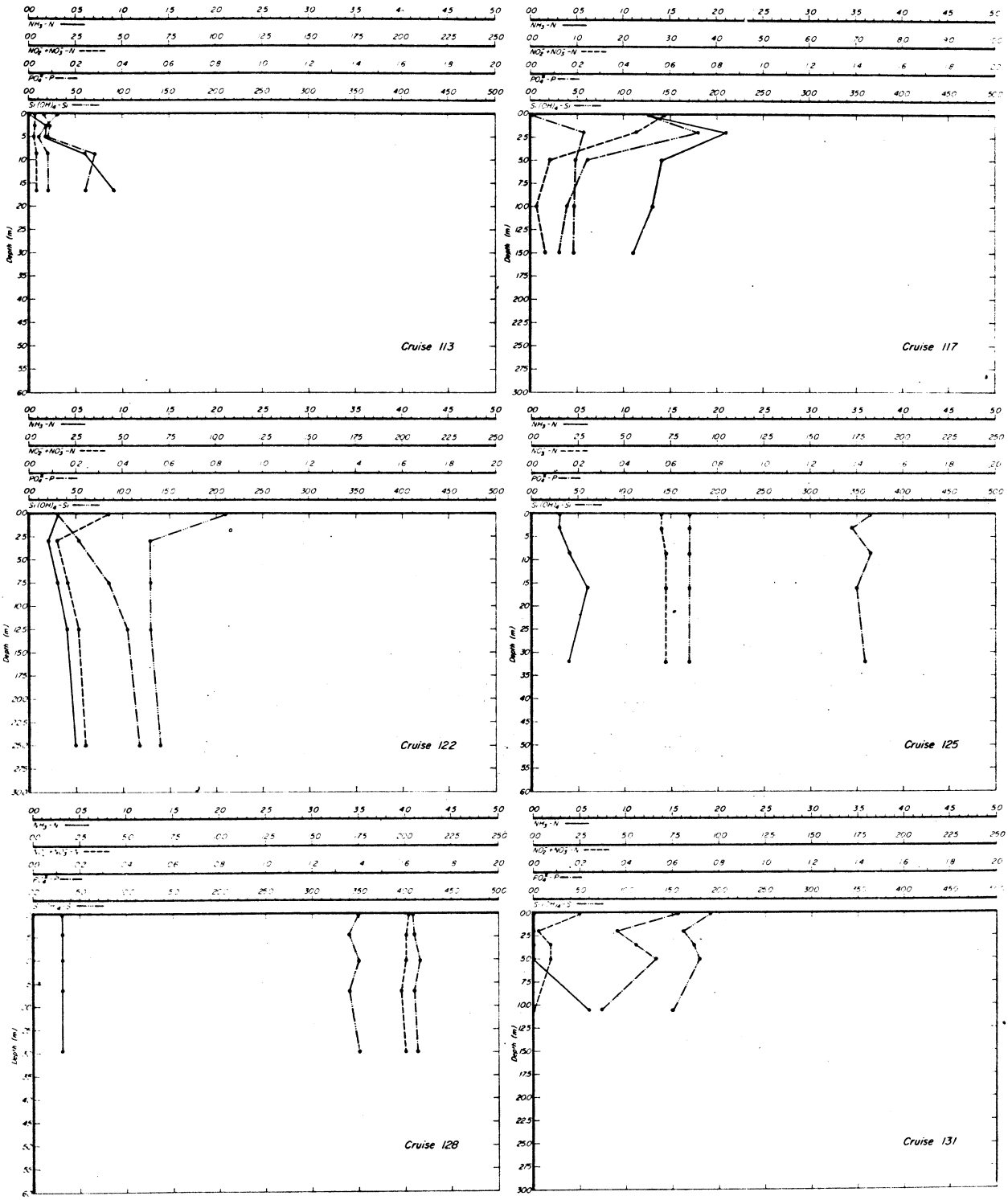
[4,5] from Goering, Shiels and Patton (In press)

Explanation

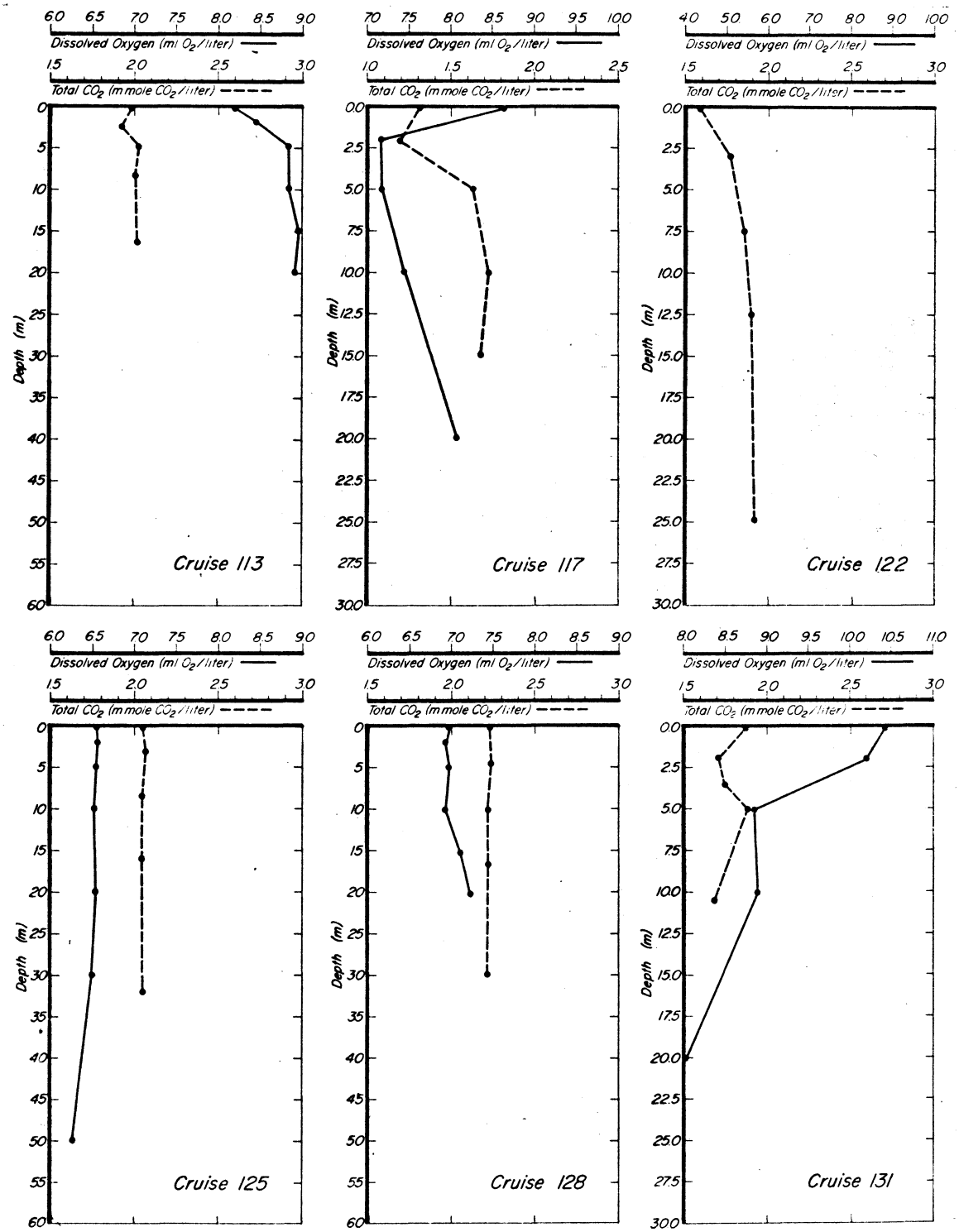
Dates of cruises made into Port Valdez, Alaska between May 1971 and April 1972.

Cruise	Date
Acona 113	16 to 26 May 1971
Acona 117	27 July to 4 Aug. 1971
Acona 122	4 to 13 Oct. 1971
Acona 125	29 Nov. to 8 Dec. 1971
Acona 128	9 to 20 Mar. 1972
Acona 131	21 to 30 Apr. 1972

Euphotic zone nutrient profiles for station 153 (Jackson Point) from *Acona* cruises 113, 117, 122, 125, 128 and 131.



Euphotic zone D. O. and ΣCO_2 profiles for station 153 (Jackson Point) from *Acona* cruises 113, 117, 122, 125, 128 and 131.



Plytoplankton standing stock and primary productivity near Jackson, Point (Station 153), Port Valdez, Alaska.

		Chlorophyll a		Phaeo-pigments		Productivity	
Cruise	Depth (m)	mg/m^3	mg/m^2	mg/m^3	mg/m^2	mgC/m^3hr	mgC/m^2hr
113	0	1.02	7.29	0.18	5.18	5.48	54.17
	2.5	0.22		1.31		5.58	
	5.1	0.89		0.14		5.80	
	8.3	0.33		0.18		2.71	
	16.7	0.23		0.04		0.13	
117	0	0.09	1.77	0.57	6.31	4.18	13.71
	2	0.19		0.64		2.72	
	5	0.15		0.52		0.40	
	10	0.09		0.29		0.19	
	15	0.06		0.24		0.07	
122	0	0.45	9.74	0.14	4.05	2.01	20.61
	3.0	0.54		0.29		2.09	
	7.5	0.49		0.24		1.24	
	12.5	0.34		0.12		0.44	
	25.0	0.28		0.09		0.01	
125	0	0.09	4.26	0.00	0.08	0.18	2.72
	3.0	0.15		0.00		0.18	
	8.5	0.10		0.00		0.15	
	16.0	0.18		0.00		0.06	
	32.0	0.09		0.01		0.00	
128	0	0.08	3.49	0.01	0.34	0.08	4.00
	4.5	0.11		0.00		0.26	
	10.0	0.11		0.00		0.16	
	16.5	0.18		0.00		0.14	
	29.5	0.05		0.05		0.03	
131	0	-	91.36	-	0	10.23	136.85
	2.0	8.82		0		18.93	
	3.5	9.31		0		20.30	
	5.0	8.71		0		16.63	
	10.5	9.08		0		1.76	

Appendix D

Carbon-14 Counting Data

from Phytoplankton and Seaweed Toxicity

Experiments

August 1971 to June 1972

Table 1: Carbon-14 uptake in toxicity vs temperature experiment, August 1971 (*Acona* cruise 117). Also see Figure 5.

Percent Crude Oil Saturated Seawater	Temperature (°C) 0	5	10	15	20
100	13.1	17.7	22.3	--	54.7
50	12.3	12.8	18.8	--	55.9
25	6.4	17.5	19.2	--	51.4
10	28.9	34.7	41.1	--	91.3
1	22.9	40.2	58.9	--	134.9
0.1	30.2	43.8	39.0	--	122.3

Table 2: Carbon-14 uptake in toxicity vs oil concentration experiment, December (*Acona* cruise 125). Also see Figure 1.

Sample		CPM
Control	Light	19.2
	Light	19.5
	Light	17.9
	Dark	9.9
10 ppm	Light	24.0
	Light	25.4
	Light	26.0
	Dark	14.2
5 ppm	Light	18.5
	Light	19.1
	Light	22.2
	Dark	10.1
1 ppm	Light	21.5
	Light	21.5
	Light	24.0
	Dark	7.8
0.5 ppm	Light	24.5
	Light	19.6
	Light	17.3
	Dark	5.5
0.1 ppm	Light	18.2
	Light	17.3
	Light	17.1
	Dark	4.2
0.05 ppm	Light	17.9
	Light	16.9
	Light	22.0
	Dark	4.5
0.01 ppm	Light	14.8
	Light	14.7
	Light	16.1
	Dark	4.6

Table 3: Carbon-14 uptake in toxicity vs exposure time experiment, December 1971 (*Acona* cruise 125). Also see Figure 9.

Sample		Incubation Time (hours)	CPM	CPM/hr
Control	Light	0.25	3.6	14.4
	Dark	0.25	4.1	16.4
Oil	Light	0.25	17.4	69.6
	Dark	0.25	20.1	80.4
Control	Light	0.5	5.1	10.2
	Dark	0.5	4.2	8.4
Oil	Light	0.5	14.7	29.4
	Dark	0.5	21.1	42.2
Control	Light	1.0	10.6	10.6
	Dark	1.0	2.6	2.6
Oil	Light	1.0	21.5	21.5
	Dark	1.0	24.2	24.2
Control	Light	2.0	12.0	6.0
	Dark	2.0	5.0	2.5
Oil	Light	2.0	19.3	9.6
	Dark	2.0	23.2	11.6
Control	Light	4.0	7.8	2.0
	Dark	4.0	7.4	1.8
Oil	Light	4.0	37.2	9.3
	Dark	4.0	44.7	11.2
Control	Light	8.0	18.4	2.3
	Dark	8.0	11.5	1.5
Oil	Light	8.0	53.6	6.7
	Dark	8.0	55.4	6.9
Control	Light	14.5	18.3	1.3
	Dark	14.5	10.9	0.8
Oil	Light	14.5	83.3	5.8
	Dark	14.5	76.6	5.3

Table 4: Carbon-14 uptake in "Dickman" experiments. Also see Figure 2.

Sample	ESR	EFF	CPM	DPM
Valdez-5-1				
Control Light	1.29	0.725	144	169
"	1.28	0.724	130	150
"	1.29	0.725	171	206
"	1.30	0.725	151	179
"	1.26	0.723	140	164
Valdez-5-1				
Control Dark	1.28	0.724	36	21
"	1.26	0.723	39	25
Valdez-5-1				
Oil Light	1.24	0.723	139	163
"	1.21	0.722	148	176
"	1.22	0.722	117	133
"	1.18	0.721	114	129
"	1.20	0.722	125	144
Valdez-5-1				
Oil dark	1.22	0.722	41	28
"	1.25	0.724	41	27
Valdez-5-2				
Control Light	1.27	0.724	267	339
"	1.26	0.723	234	292
"	1.26	0.723	279	357
"	1.27	0.724	226	282
"	1.26	0.723	209	260
Valdez-5-2				
Control Dark	1.26	0.724	45	33
"	1.27	0.724	40	26
Valdez-5-2				
Oil Light	1.25	0.723	256	325
"	1.28	0.725	292	373
"	1.23	0.722	254	322
"	1.23	0.722	247	312
"	1.26	0.724	299	384
Valdez-5-2				
Oil Dark	1.24	0.723	48	37
"	0.92	0.710	44	33
Valdez-5-3				
Control Light	1.26	0.724	687	920
"	1.27	0.724	725	972
"	1.23	0.722	888	1201
"	1.25	0.723	691	927
"	1.26	0.724	800	1096
Valdez-5-3				
Control Dark	1.26	0.724	57	50
"	1.26	0.724	62	57

Table 4 Con't.

Sample	ESR	EFF	CPM	DPM
Valdez-5-3				
Oil Light	1.08	0.719	765	1034
"	1.10	0.720	710	957
"	0.80	0.689	717	1011
"	1.10	0.720	802	1084
"	0.71	0.655	733	1088
Valdez-5-3				
Oil Dark	0.67	0.633	49	43
"	1.22	0.722	51	42
Valdez-5-4				
Control Light	1.29	0.725	460	605
"	1.28	0.725	451	592
"	1.28	0.725	434	569
"	1.27	0.724	466	615
"	1.28	0.725	478	631
Valdez-5-4				
Control Dark	1.26	0.723	37	22
"	1.28	0.725	33	17
Valdez-5-4				
Oil Light	1.26	0.723	513	680
"	1.22	0.722	439	579
"	1.27	0.724	467	615
"	1.20	0.722	465	613
"	1.19	0.722	495	656
Valdez-5-4				
Oil Dark	1.22	0.722	37	22
"	0.14	0.251	30	35
Valdez-6-3				
Control Light	1.25	0.835	3018	3589
"	1.24	0.835	3972	4732
"	1.26	0.835	3428	4080
"	1.24	0.835	3090	3675
"	1.24	0.835	3790	4514
Valdez-6-3				
Control Dark	1.27	0.835	146	151
"	1.25	0.835	157	163
Valdez-6-3				
Oil Light	1.00	0.835	1471	1736
"	1.10	0.835	1038	1217
"	0.96	0.835	1140	1340
"	--	--	--	--
"	1.12	0.835	1540	1820
Valdez-6-3				
Oil Dark	1.22	0.835	610	705
"	1.17	0.835	483	554

Table 5: Carbon-14 uptake in toxicity vs temperature experiment, March 1972 (*Acona* cruise 128). Also see Figure 6.

Sample	Temp.	ESR	EFF	CPM	DPM
Control Light					
V-5-6	5C	1.27	0.724	127	146
"	5C	1.28	0.725	95	101
"	5C	1.29	0.725	125	143
Control Dark					
V-5-6	5C	1.30	0.725	67	63
Oil Light					
V-5-6	5C	1.19	0.722	60	54
"	5C	1.20	0.722	99	108
"	5C	1.19	0.722	88	92
Oil Dark					
V-5-6	5C	--	--	--	--
Control Light					
V-5-6	10C	1.30	0.725	114	129
"	10C	1.30	0.725	155	185
"	10C	1.28	0.725	150	177
Control Dark					
V-5-6	10C	1.30	0.725	94	101
Oil Light					
V-5-6	10C	1.21	0.723	65	61
"	10C	1.20	0.722	79	80
"	10C	1.21	0.723	81	82
Oil Dark					
V-5-6	10C	1.20	0.722	39	25
Control Light					
V-5-6	15C	1.30	0.725	113	126
"	15C	1.31	0.726	113	126
"	15C	1.30	0.725	115	129
Control Dark					
V-5-6	15C	1.29	0.725	74	72
Oil Light					
V-5-6	15C	1.20	0.722	53	45
"	15C	1.20	0.722	71	69
"	15C	1.22	0.723	73	71
Oil Dark					
V-5-6	15C	1.20	0.722	42	28
Control Light					
V-5-6	20C	1.29	0.725	127	146
"	20C	1.30	0.725	103	114
"	20C	1.29	0.725	90	95
Control Dark					
V-5-6	20C	1.30	0.725	118	133
Oil Light					
V-5-6	20C	1.23	0.723	58	51
"	20C	1.21	0.723	50	40

Table 5 Con't.

Sample	Temp.	ESR	EFF	CPM	DPM
Oil Light V-5-6	20C	1.21	0.722	65	61
Oil Dark V-5-6	20C	1.22	0.722	43	30

Table 6: Carbon-14 uptake in toxicity vs temperature experiment, April 1972 (*Acona* cruise 131). Also see Figure 6.

Sample	Temp.	ESR	EFF	CPM	DPM
Control Light					
V-6-6	3C	1.26	0.835	357	402
"	3C	1.28	0.835	341	383
"	3C	1.27	0.835	339	380
Control Dark					
V-6-6	3C	1.28	0.835	72	61
Oil Light					
V-6-6	3C	1.26	0.835	391	443
"	3C	1.24	0.835	316	354
"	3C	1.25	0.835	338	380
Oil Dark					
V-6-6	3C	1.24	0.835	75	65
Control Light					
V-6-6	5C	1.28	0.835	850	992
"	5C	1.27	0.835	852	995
"	5C	1.29	0.835	706	820
Control Dark					
V-6-6	5C	1.29	0.835	298	332
Oil Light					
V-6-6	5C	1.23	0.835	665	771
"	5C	1.24	0.835	592	684
"	5C	1.24	0.835	463	529
Oil Dark					
V-6-6	5C	1.23	0.835	277	306
Control Light					
V-6-6	10C	1.26	0.835	1111	1306
"	10C	1.26	0.835	889	1039
"	10C	1.25	0.835	1018	1194
Control Dark					
V-6-6	10C	1.29	0.835	27	7
Oil Light					
V-6-6	10C	1.25	0.835	833	972
"	10C	1.26	0.835	744	866
"	10C	1.25	0.835	718	835
Oil Dark					
V-6-6	10C	1.25	0.835	294	327
Control Light					
V-6-6	15C	1.28	0.835	885	1034
"	15C	1.28	0.835	1026	1204
"	15C	1.30	0.835	635	735
Control Dark					
V-6-6	15C	1.28	0.835	355	400
Oil Light					
V-6-6	15C	1.21	0.835	835	974
"	15C	1.23	0.835	857	1002
"	15C	1.25	0.835	860	1005

Table 6 Con't.

Sample	Temp.	ESR	EFF	CPM	DPM
Oil Dark					
V-6-6	15C	1.22	0.835	306	341
Control Light					
V-6-6	20C	1.27	0.835	771	899
"	20C	1.27	0.835	779	907
"	20C	1.27	0.835	464	530
Control Dark					
V-6-6	20C	1.28	0.835	235	256
Oil Light					
V-6-6	20C	1.23	0.835	420	478
"	20C	1.22	0.835	524	602
"	20C	1.16	0.835	296	329
Oil Dark					
V-6-6	20C	1.22	0.835	392	444

Table 7: Carbon-14 uptake in toxicity vs oil concentration experiment, April 1972 (*Acona* cruise 131). Also see Figure 3.

Sample	Oil Concentration	ESR	EFF	CPM	DPM
Oil Light V-6-5	50%	1.26	0.835	753	876
Oil Dark V-6-5	50%	1.26	0.835	51	36
Oil Light V-6-5	5%	1.22	0.835	996	1167
Oil Dark V-6-5	5%	1.27	0.835	90	82
Oil Light V-6-5	0.5%	1.24	0.835	1368	1613
Oil Dark V-6-5	0.5%	1.28	0.835	78	68
Oil Light V-6-5	0.05%	1.25	0.835	1532	1809
Oil Dark V-6-5	0.05%	1.27	0.835	79	69
Control Light V-6-5	0	1.26	0.835	1154	1357
Control Light V-6-5	0	1.26	0.835	1134	1332
Control Dark V-6-5	0	1.27	0.835	93	86
Control Dark V-6-5	0	1.26	0.835	114	111

Table 8: Carbon-14 uptake in toxicity vs light intensity experiment, April 1972 (*Acona* cruise 131). Also see Figure 7.

Sample	Oil Concentration	Light Intensity	ESR	EFF	CPM	DPM
Treated V-6-7	50%	100%	1.25	0.835	1199	1410
		50%	1.24	0.835	1748	2068
		25%	1.27	0.835	2596	3084
		10%	1.27	0.835	1652	1953
		1%	1.26	0.835	82	73
		(Dark) 0%	1.27	0.835	68	56
Treated V-6-7	5%	100%	1.25	0.835	2513	2985
		50%		0.835	2678	3182
		25%		0.835	1624	1920
		10%		0.835	1995	2364
		1%		0.835	124	124
		(Dark) 0%		0.835	139	141
Treated V-6-7	0.5%	100%	1.26	0.835	2626	3119
		50%	1.26	0.835	2487	2953
		25%	1.22	0.835	2679	3183
		10%	1.24	0.835	2038	2415
		1%	1.25	0.835	161	167
		(Dark) 0%	1.25	0.835	105	101
Control V-6-7	0%	100%	1.25	0.835	3212	3822
		50%	1.25	0.835	3104	3693
		25%	1.26	0.835	3021	3592
		10%	1.27	0.835	2233	2648
		1%	1.25	0.835	108	104
		(Dark) 0%	1.24	0.835	89	81

Table 9: Carbon-14 uptake in toxicity vs oil concentration experiment, June 1972. Also see Figure 4.

Sample	Oil Concentration	ESR	EFF	CPM	DPM
Oil Light	50%	1.19	0.834	2,737	3,282
Oil Dark	50%	1.19	0.834	151	181
Oil Light	25%	1.19	0.834	7,757	9,301
Oil Dark	25%	1.18	0.834	223	267
Oil Light	5%	1.20	0.834	10,158	12,180
Oil Dark	5%	1.20	0.834	258	310
Oil Light	2.5%	1.21	0.834	9,080	10,887
Oil Dark	2.5%	1.23	0.834	271	325
Oil Light	0.5%	1.20	0.834	13,297	15,944
Oil Dark	0.5%	1.21	0.834	230	276
Oil Light	0.25%	1.21	0.834	12,010	14,400
Oil Dark	0.25%	1.28	0.834	--	--
Oil Light	0.05%	1.27	0.834	29,684	35,592
Oil Dark	0.05%	1.21	0.834	548	657
TB Light	5.5 ppm	1.03	0.834	9,873	11,838
	5.5 ppm	1.29	0.834	--	--
TB Dark	5.5 ppm	1.07	0.834	560	672
	5.5 ppm	1.11	0.834	565	678
Control Light	0%	1.20	0.834	14,127	16,939
	0%	1.20	0.834	14,988	17,971
Control Dark	0%	1.21	0.834	1,118	1,341
	0%	1.21	0.834	1,534	1,839

Table 10: Carbon-14 uptake in seaweeds in oil toxicity experiments, March 1972. Also see Figure 13.

Sample		Oil Concentration	DPM	Dry Weight (mg)	DPM mg
Fucus	L	50%	16,319	2.85	5,726
V-5-5	D	50%	2,303	3.70	622
	L	5%	20,692	3.76	5,503
	D	5%	1,332	2.74	486
	L	0.5%	32,575	6.91	4,714
	D	0.5%	2,930	6.20	473
	L	0.05%	39,879	8.10	4,923
	D	0.05%	2,489	4.56	546
	L	0	31,800	6.24	5,096
	L	0	--	--	--
	D	0	4,077	9.56	426
	D	0	--	--	--
Alaria	L	50%	216,507	8.20	26,403
V-5-5	D	50%	1,521	2.21	688
	L	5%	2,596	0.05	51,920
	D	5%	938	1.66	565
	L	0.5%	31,221	1.21	25,802
	D	0.5%	1,144	3.19	359
	L	0.05%	98,569	3.02	32,639
	D	0.05%	450	1.04	433
	L	0	48,290	1.48	32,628
	L	0	61,465	2.31	26,608
	D	0	1,536	3.42	449
	D	0	2,726	4.61	591

Table 11: Carbon-14 uptake in seaweeds in oil toxicity experiments, April 1972. Also see Figure 13.

Sample		Oil Concentration	DPM	Dry Weight (mg)	DPM mg
Laminaria	L	50%	3,379	0.68	4,969
V-6-2	D	50%	1,177	1.47	801
	L	5%	15,204	2.22	6,849
	D	5%	859	4.73	182
	L	0.5%	11,416	2.27	5,029
	D	0.5%	218	1.44	151
	L	0.05%	15,113	2.03	7,445
	D	0.05%	850	3.94	216
	L	0	21,250	2.16	9,838
	L	0	12,949	1.39	9.316
	D	0	477	1.43	334
	D	0	515	3.08	167
Costaria	L	50%	18,014	1.70	10,596
V-6-2	D	50%	1,163	1.60	727
	L	5%	50,072	4.72	10,608
	D	5%	502	2.72	185
	L	0.5%	14,700	1.89	7,778
	D	0.5%	529	2.82	188
	L	0.05%	39,360	2.68	14,687
	D	0.05%	946	3.36	282
	L	0	43,444	3.80	11,433
	L	0	16,200	2.10	7,714
	D	0	457	1.47	311
	D	0	338	1.43	236
Ulva	L	50%	49,409	4.43	11,153
V-6-2	D	50%	3,071	2.79	1,101
	L	5%	83,563	2.99	27,948
	D	5%	458	4.84	95
	L	0.5%	56,521	3.43	16,478
	D	0.5%	401	4.53	88
	L	0.05%	43,281	2.85	15,186
	D	0.05%	327	2.77	118
	L	0	56,648	3.26	17,377
	L	0	72,377	4.69	15,432
	D	0	465	3.28	142
	D	0	326	3.31	98
Halosaccion	L	50%	13,643	1.72	7,932
V-6-4	D	50%	206	1.70	121
	L	5%	12,315	1.62	7,602
	D	5%	143	0.68	210
	L	0.5%	8,580	0.63	13,619
	D	0.5%	196	0.93	211
	L	0.05%	60,358	6.13	9.846
	D	0.05%	830	6.81	122

Table 11 Con't.

Sample		Oil Concentration	DPM	Dry Weight (mg)	DPM mg
Halosaccion	L	0	57,808	8.18	7,067
V-6-4			64,109	7.94	8,074
(con't.)	L	0	39,070	4.37	8,940
	D	0	549	5.60	98
	D	0	851	7.81	109
Rhodymenia	L	50%	22,146	3.30	6,711
Y-6-4	D	50%	730	6.69	109
			1,216	10.28	118
	L	5%	94,943	9.27	10,242
	D	5%	1,192	7.58	157
	L	0.5%	50,380	5.54	9,094
	D	0.5%	1,022	9.30	110
	L	0.05%	78,365	9.79	8,005
			75,491	9.11	8,287
			82,387	9.85	8,364
			73,950	10.36	7,138
			74,078	9.38	7,897
	D	0.05%	686	7.94	86
	L	0	89,190	9.07	9,834
	L	0	80,620	10.20	7,904
	D	0	661	6.03	110
	D	0	1,700	8.29	205
Cladophora	L	50%	21,286	2.53	8,413
Y-6-4	D	50%	569	7.16	80
	L	5%	75,070	4.61	16,284
	D	5%	264	6.58	40
	L	0.5%	151,378	6.31	23,990
	D	0.5%	614	9.10	68
	L	0.05%	145,523	7.46	19,507
	D	0.05%	1,099	9.69	113
	L	0	265,924	11.65	22,826
	L	0	174,106	7.04	24,731
	D	0	457	7.43	62
	D	0	678	6.49	104